SCIENTIFIC PROCEEDINGS.

Vol. XXVI. March, 1929. No. 6.

Peking Branch.

Peking Union Medical College, January 24, 1929.

4322

Effect of Neoarsphenamin on the Number of Blood Platelets.

JUI-WU-MU. (Introduced by H. A. Reimann.)

From the Division of Dermatology and Syphilology of the Department of Medicine, Peking Union Medical College, Peping, China.

The blood platelets show characteristic variations in number after the intravenous injection of certain toxic substances and during certain acute infectious diseases. At first, following the injection of the substance or during the febrile period of the disease the number of platelets is diminished. The period of thrombopenia is then usually followed by a rapid rise to the normal number (350,000 per cu. mm.) and often by a period of thrombocytosis, the number occasionally reaching 750,000 per cu. mm. or more.

It has been suggested that thrombosis may occur more readily as a result of the excessive number of platelets, although they are not the sole factor in the formation of thrombi.[1] Hartmann[2] has pointed out that the appearance of thrombosis which occurs during convalescence from typhoid fever coincides with the period of thrombocytosis. Recently a case in this hospital of cerebral thrombosis developing 5 days after the intravenous injection of 0.6 gm. of neoarsphenamin suggested the possibility of a similar relationship, although platelet counts were not made at the time.

Several investigators have observed the immediate diminution of the number of platelets following arsphenamin therapy and numerous cases of purpura occurring after arsphenamin treatment are recorded. However, little or no attention has been given to the later period when thrombocytosis may occur. The following observations were made to determine whether or not thrombocytosis follows the period of thrombopenia subsequent to the intravenous injection of neoarsphenamin (novarsenobenzol "Billon").

---

[1] Lee, R. I., Minot, G. R., and Vincent, B., *J. Am. Med. Assn.*, 1916, lxvii, 719.

[2] Hartmann, E., *Deutsch. Arch. Klin. Med.*, 1927, clviii, 1.

The effect of the injection of the drug on the number of platelets of 14 patients was studied. The usual dosage given was 0.1 g. per kg. of body weight. The direct method of counting[3] was employed. Counts were made before the injection and then at intervals of from 10 minutes to 1 day after the injection for 5 days.

In 4 cases the number of platelets did not vary beyond the range of experimental error (±25,000). However, in agreement with other reports, there was a diminution in the number of platelets (170,000 to 300,000 per cu. mm.) from 10 to 30 minutes after injection in 10 cases. In 5 of these patients, flushing of the face, dizziness, nausea, chilliness and fever preceded the diminution in number. After a period of about 6 hours the number of platelets returned to normal and a period of slight overproduction followed which lasted from 1 to 3 days. The platelets during the period of thrombocytosis never exceeded 450,000 per cu. mm. in any case. A

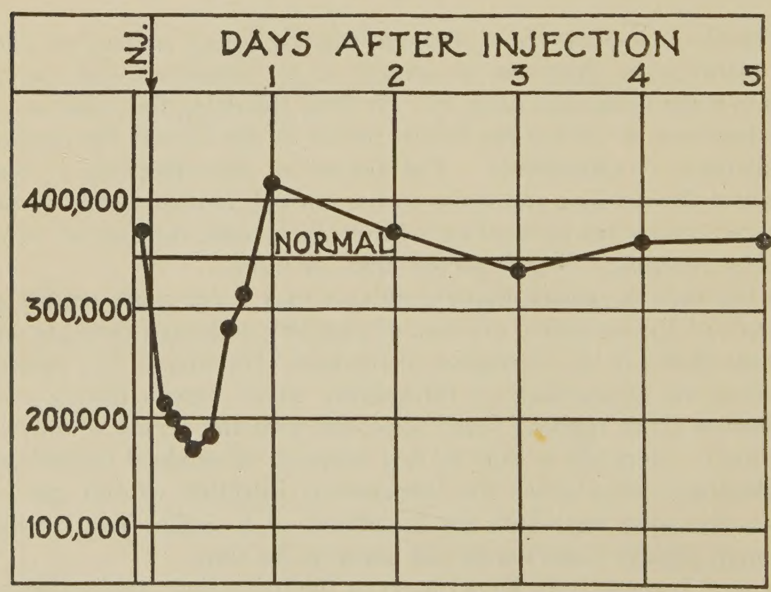


CHART 1.
Variations in the number of platelets following neoarsphenamin injection.

---

[3] Reimann, H. A., *J. Exp. Med.*, 1924, xl, 553.

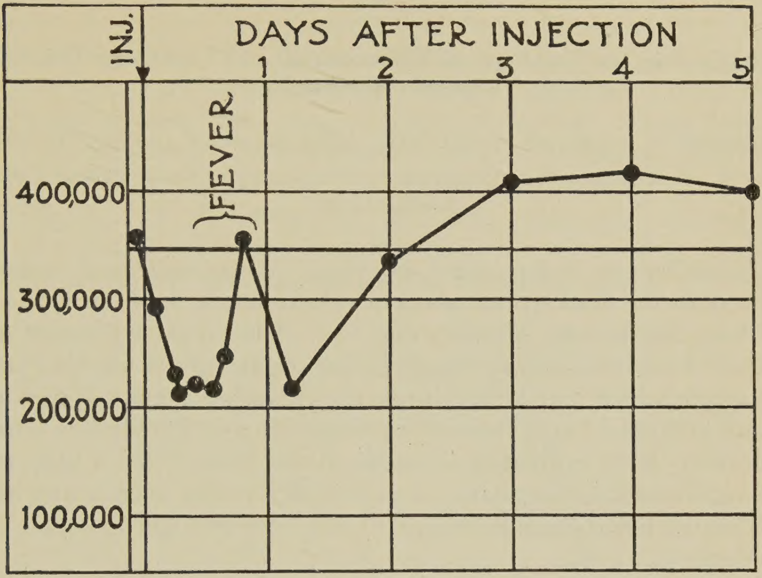


CHART 2.

The effect of a febrile reaction on the number of platelets following neoarsphenamin injection.

typical curve is illustrated in Chart 1. In 2 cases the diminution did not occur until 24 hours after injection. But in these 2 cases fever (38° to 38.5° C.) which appeared 12 hours after injection preceded the diminution. Chart 2 shows the effect of a febrile reaction occurring in another case 8 hours after the injection. The fever apparently caused a second diminution in the number of platelets which was followed in 2 days by thrombocytosis.

Summary: The number of blood platelets was diminished following the intravenous injection of neoarsphenamin in 10 out of 14 patients. The period of thrombopenia was followed by slight thrombocytosis. The number of platelets did not exceed 450,000 per cu. mm. in any of the cases observed. The variations in the number of platelets were usually greater in patients developing a febrile reaction after treatment. It would seem that only in the exceptional case does a reaction occur which is severe enough to provoke a marked thrombopenia and a subsequent marked thrombocytosis.

4323

Studies on the Etiology of Splenomegaly of Unknown Origin. (Splenic Mycosis?)

HOBART A. REIMANN, TIMOTHY G. KUROTCHKIN AND EDITH TSO.

From the Departments of Medicine and Pathology, Peking Union Medical College, Peping, China.

Recent reports from widely separated sources indicate a renewed interest in the etiology of primary splenomegaly with cirrhosis of the liver and anemia (Banti's disease). Gibson[1] and Fawcett and Gibson[2] have isolated a streptothrix-like organism (Nocardia) from the spleen which they believe to be the causative agent. Pinoy and Nanta in Algiers[3] and Emile-Weil, Gregoire and Flandrin in Paris[4] and others have cultivated a fungus, Aspergillus, from a high percentage of spleens from cases of splenomegaly with anemia and have applied the terms "splenic mycosis" and "mycotic splenomegaly" to the disease.

These views have not been generally accepted. Although Oberling[5] and Jaffe and Hill[6] were convinced that certain thread-like elements found in the siderotic nodules in the spleen were mycotic filaments, in harmony with the interpretations set forth by other investigators, they found the same structures in other diseases as well, *f. i.*, leukemia, sickle cell anemia, etc. They suggested that the fungus was a secondary invader in a previously damaged spleen. Other observers, Gamna,[7] Langeron[8] and da Fonseca and da Area Leao[9] believed the so-called mycelium found in the nodules to be degenerated tissue fibers and that no connection between the fungus and the disease exists.

In northern China splenomegaly with cirrhosis of the liver, ascites, anemia and leucopenia is a common disease. A clinical study of this group of cases will be published elsewhere. We have recently had the opportunity of examining 4 spleens and portions of liver from 3 cases of splenomegaly and from 1 case of primary cirrhosis of the liver. The specimens were removed by operation.

---

[1] Gibson, A. G., *Quart. J. Med.*, 1914, vii, 153.

[2] Fawcett, J., and Gibson, A. G., *Lancet*, 1928, l, 1171

[3] Pinoy, E., and Nanta, A., *Compt. Rend. Soc. Biol.*, 1927, xcvii, 67.

[4] Emile-Weil, Gregoire and Flandrin, *Bull. et Mem. Soc. Med. d. Hôp.*, 1927, No. 17, 713.

[5] Oberling, Ch., *Presse Med.*, 1928, i, 2.

[6] Jaffe, R. H., and Hill, L. R., *Arch. Path.*, 1928, vi, 196.

[7] Gamna, C., *Presse Med.*, 1928, xxiii, 357.

[8] Langeron, M., *Ann. Parasitol. Hum. et Comp.*, 1928, vi, 211.

[9] Da Fonseca, O., and de Area Leao, A. E., *Suppl. das Mem., Inst. Oswaldo Cruz*, 1928, i, 16.

By inoculating the crushed splenic pulp on Sabouraud's medium we were able to cultivate a fungus from each spleen. Tubes inoculated with portions of liver remained sterile. The fungus derived from the first case was classified as an uncommon form of Penicillium (Fig. 1). It was pathogenic for mice, guinea pigs and rabbits. The fungus recovered from the other 3 spleens (Fig. 2) is still unclassified and does not conform to the description of any of

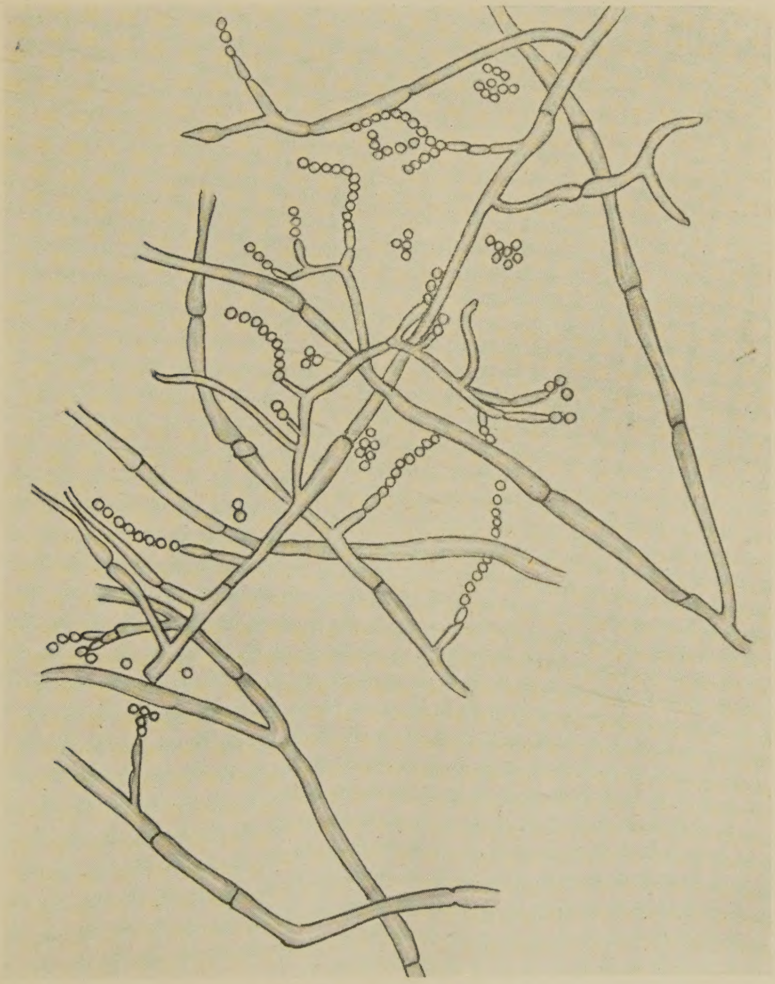


FIG. 1.
Camera lucida drawing of a microculture of Penicillium derived from Case 1. Methylene blue stain, about X 760.

the varieties recovered by the other investigators. It was not pathogenic for laboratory animals. It grows readily at 37° C. and forms large snow white colonies which became dark brownish with age. The growth is aerial and does not penetrate into the medium. In microcultures the filaments are from 1.5 to 2 microns thick. The mycelial threads are segmented and show slight constrictions at each joint. Large conidia are born directly upon the terminal ends of the hyphae. Thick, slightly curved, detached bodies appear to be the spores of the fungus.

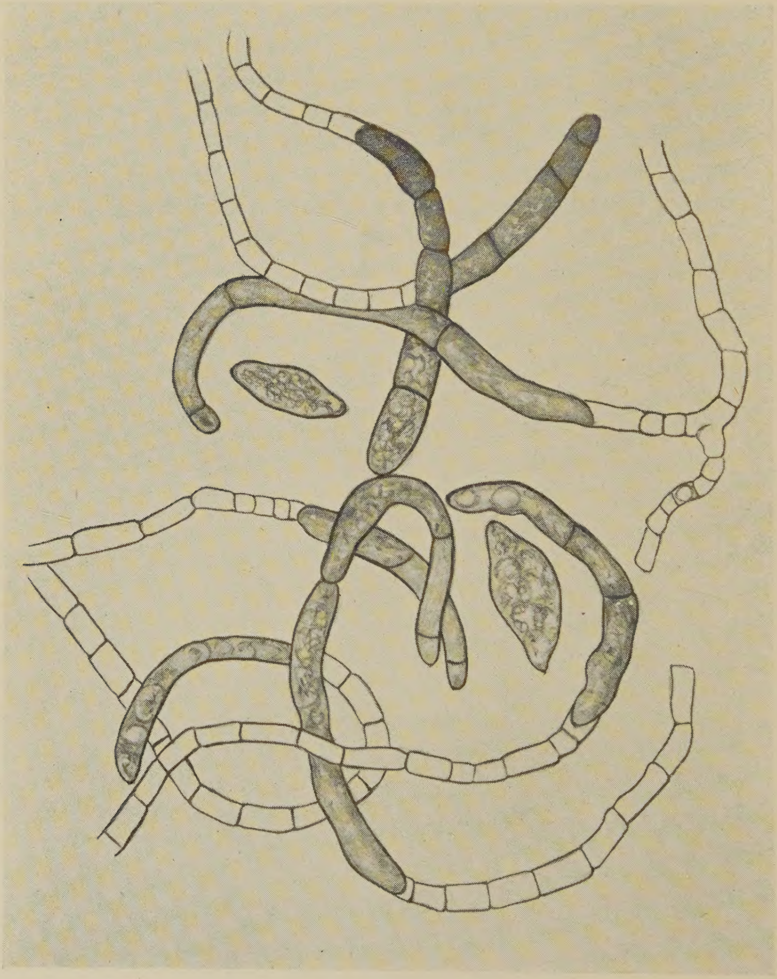


FIG. 2.

Camera lucida drawing of a microculture of the fungus derived from Cases 2, 3 and 4. Methylene blue stain, about X 1500.

Cultures of spleen and liver on other types of media to detect the presence of bacteria or other organisms remained sterile.

Experiments to ascertain the relationship between primary splenomegaly and the presence of the fungus in the spleen are in progress.

4324

Filaments in Siderotic Nodules of Spleen in Cases of Splenomegaly of Unknown Origin.

C. H. HU, HOBART A. REIMANN and TIMOTHY G. KUROTCHKIN.

From the Departments of Pathology and Medicine, Peking Union Medical College, Peping, China.

In the recent studies concerning primary splenomegaly, much attention has been given to the presence in the spleen of the siderotic, so-called Gandi-Gamna nodules. Several observers[1, 2, 3, 4, 5] claimed that these siderotic or iron pigment deposits in the spleen were the direct result of the growth of a fungus. They considered the thread-like structures found in the nodules to be mycelium and were able to recognize club-like branches and fructification organs. The cultivation of fungi from a number of spleens from cases of primary splenomegaly seemed to confirm these views. On the other hand, many of the German pathologists since 1920 as well as several recent investigators[6, 7] believed that the filamentous structures composing the pigment deposits were degenerated tissue fibers.

We have made histological examinations of the spleens removed at operation from cases of primary splenomegaly and have compared the filaments found in the siderotic nodules as described by others, with the two varieties of fungi cultivated from the same spleens in this laboratory.[8] Upon close examination of sections stained in the usual manner certain structures were found in the

---

[1] Gibson, A. G., *Quart. J. Med.*, 1914, vii, 153.

[2] Fawcett, J., and Gibson, A. G., *Lancet*, 1928, i, 1171.

[3] Emile-Weil, P., Gregoire, R., and Flandrin, P., *Bull. et Mem. Soc. Med. d. Hôp.*, 1927, No. 17, 713.

[4] Emile-Weil, P., Gregoire, R., and Flandrin, P., *Le Sang*, 1927, i, 509.

[5] Jaffe, R. H., and Hill, L. R., *Arch. Path.*, 1928, vi, 196.

[6] Gamna, C., *Presse Med.*, 1928, No. 23, 357.

[7] Da Fonseca, O., and de Area Leao, A. E., *Suppl. d. Mem., Inst. Oswaldo Cruz*, 1928, No. 1, 16.

[8] Reimann, H. A., Kurotchkin, T. G., and Tso, E., Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 410.

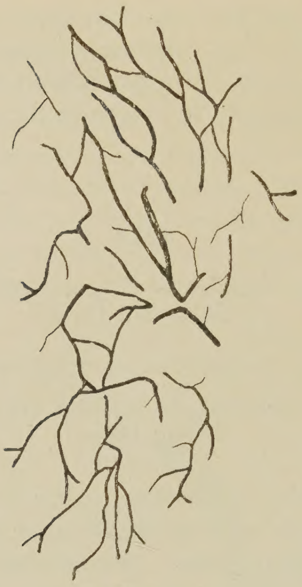


FIG. 1.

Wavy, branching fibers as they appeared in a section of a siderotic nodule. Hematoxylin and eosin stain. Camera lucida drawing, X 500.

pigmented areas which could be readily mistaken for the mycelium of a fungus. Two kinds of fibers were often present in the same area. One form appeared like wavy, branching threads of varying width (Fig. 1). The other kind, which was less likely to be mistaken for mycelium was composed of wider, more irregular, heavily pigmented rods (Fig. 2). The rods appeared to be irreguarly segmented and the ends showed jagged edges suggesting fracture surfaces of brittle fibers. Structures resembling conidia were also occasionally found in the nodules. They proved to be deposits of calcium and iron pigment. However, the appearance of the thread-like structures was different from the 2 varieties of fungi isolated from the same spleens[8] (Figs. 1 and 2). Moreover, siderotic nodules with similar filaments were found in spleens from cases of malaria, tuberculosis, kala-azar and syphilis as well as in an adenomatous thyroid gland.

When sections of spleen containing the siderotic nodules were stained with Mallory's method for iron[9] the wavy, branched filaments in the nodules were stained bright blue indicating the presence of iron. When similar sections were stained with Weigert's elastic tissue stain the normal elastic fibers around the nodules stained

---

[9] Mallory and Wright, *Path. Tech.*, 8th Ed., p. 209.

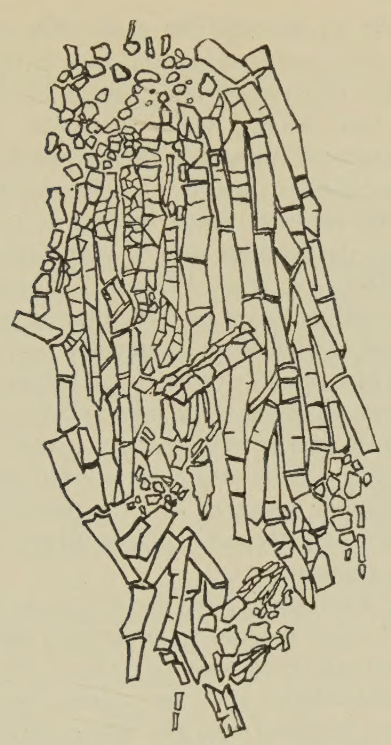


FIG. 2.
Large, brittle, rod-like fibers found in a siderotic nodule. Unstained, teased specimen. Camera lucida drawing, X 500.

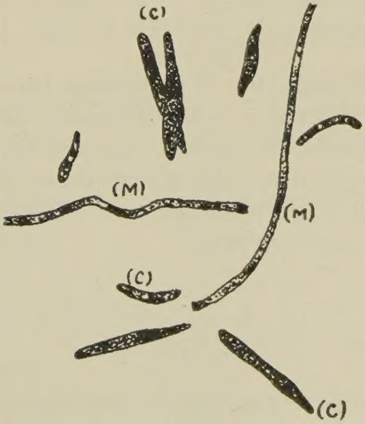


FIG. 3.
Appearance of an unidentified fungus, recovered from 3 cases of splenomegaly, after injection into normal splenic tissue. (M) mycelium; (C) conidia or spores. Camera lucida drawing, X 500 from a section stained with Gram-Weigert's method.

blue-black. Finally by staining the same slide first with Mallory's method (without the counterstain) and then with Weigert's method it was observed that the normal bluish black elastic fibers were continuous with the iron bearing wavy filaments. In other words it appeared that the wavy filaments in the nodule were actually degenerated elastic tissue fibers in which iron pigment had been deposited.

Similar observations were made in regard to the wider, brittle rod-like structures. In sections stained with hematoxylin and eosin, normal collagen fibers are red. Within a siderotic nodule, normal collagen fibers as well as green or dark blue structures of a similar contour were found, which were frequently segmented and broken. It was possible to observe all grades of pigmentation from a fine or a coarse heavy green stippling of collagen fibers to fibers which were loaded with pigment and stained solidly green or blue. In some instances the gradation from stippling to solid staining was seen in the same fiber. Thus it appeared that the coarse rod-like structures in the nodules were degenerated collagen fibers which had become impregnated with pigment.

Similar staining methods applied to the 2 varieties of fungi recovered from cases of splenomegaly[8] gave entirely different pictures. To demonstrate the actual appearance of one of the fungi ([8], Fig. 2) when artificially introduced into the spleen, heavy suspensions in saline solution were injected into the splenic pulp of 3 dogs. The spleens were removed 1 hour, 1 day and 1 month respectively after injection. No traces of the fungus were found in the spleen removed after 1 month. The morphology of the fungus as it appeared in the tissue of the other spleens (illustrated in Fig. 3) did not resemble the fibers found in the siderotic nodules.

Conclusions: Although the relationship between certain fungi and primary splenomegaly is still undecided we feel that the filaments found in the siderotic nodules are not mycotic hyphae since (a) their origin can be traced to normal tissue fibers and (b) their staining reactions and morphology are different from the fungi cultivated from the same spleens.

4325

Difference in Reticulocyte Behavior in Anemia from Malaria and in Pernicious Anemia.

C. S. YANG AND HILDING BERGLUND.

From the Department of Medicine, Peking Union Medical School, Peping, China.

Until the well known results in the treatment of pernicious anemia were obtained by the feeding of liver or liver extract, the hemolytic features of this disease as presented by both skin and plasma were generally considered of sufficient significance to classify pernicious anemia essentially as a form of hemolytic anemia. This assumption logically necessitates further assumptions: that in the severe stage of the disease with an erythrocyte count of about 1,000,000 and marked yellowness of skin and plasma the blood destruction is marked. Since such a low level might be maintained for months, a rapid regeneration would be necessary to compensate the rapid destruction. If so, a high reticulocyte count would be expected under these very circumstances. Contrary to such an expectation the reticulocytes are usually found below half of 1%.

This paper aims to contribute to the argument against pernicious anemia as essentially a hemolytic anemia. We demonstrate in the observation here presented (Chart 1) that in one truly hemolytic

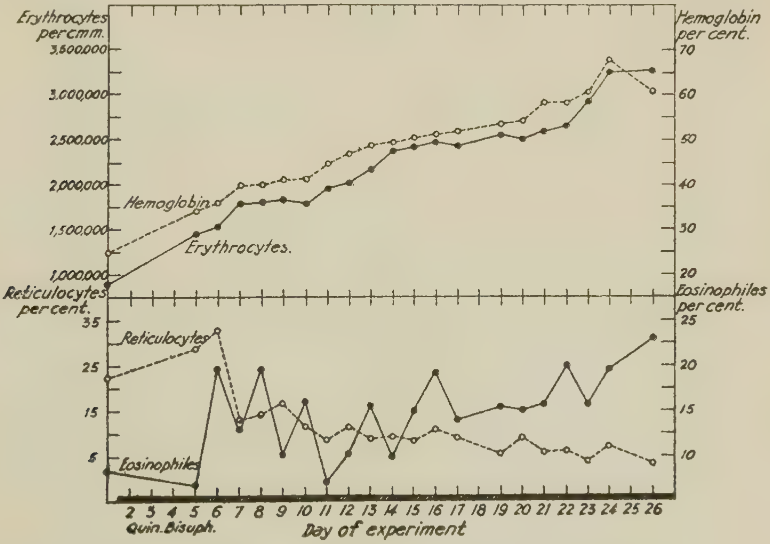


CHART 1.

Difference in reticulocyte behavior in anemia from malaria and in pernicious anemia.

anemia in man, due to untreated tertian malaria, at an erythrocyte level of less than 1,000,000 there is a lively blood regeneration going on, as expressed by the number of reticulocytes reaching 23% of the total number of erythrocytes. The degree of regeneration indicated by this reticulocyte count was needed approximately to keep pace with the destruction. This blood picture closely resembles the one produced in dogs by means of phenyl hydrazine poisoning[1] but is fundamentally different from the one in an untreated case of pernicious anemia. The mere stopping of the malaria by quinine without any treatment of the anemia in this case sufficed to produce recovery, incidentally making the picture fairly identical with the regeneration phase in the phenyl hydrazine experiment referred to and also to the reaction produced by liver extract in pernicious anemia.

Our observations reveal one other interesting feature: the marked eosinophilia which developed in this case as soon as recovery set in or as soon as the treatment of the malaria was begun. Similar eosinophilia was observed in pernicious anemia when treated with liver.

The reason for presenting this observation is the absence of cases like this in most clinics where the anemia problem is being studied at present.

4326

Deficiency Anemia in Chinese, Responding to Cod Liver Oil.

HILDING BERGLUND, CHESTER S. KEEFER AND CHI S. YANG.

From the Department of Medicine, Peking Union Medical College, Peping, China.

In attempting to survey the anemia situation in North China several factors presented themselves to us as significant. (1) Even outside the so-called famine areas food deficiencies are common, particularly qualitative deficiencies. This point is elucidated by the work of Hsien Wu[1] and his collaborators, who showed that the North China diet is low in fat and on the verge of fat soluble vitamin deficiency. (2) Attacks of prolonged diarrhea, due to dysentery or to yet unknown causes, in a population living on such a diet are apt especially to bring out 2 groups of symptoms: of anemia and of hydrops. (3) We were impressed with the fact that severe secondary anemias of unknown origin, when hospitalized, showed a seemingly spontaneous recovery in the course of about 2 months. Such anemias of lesser severity were also seen during pregnancy, on proper diet already showing marked improvement before term. These anemias are hematologically characterized by a color index in the neighborhood of unity, of the absence of anisocytosis or poikilocytosis, of the absence of nucleated red cells and of immature granulocytes and of practically normal white cell formula. There are no hemolytic features. These anemias show free hydrochloric acid in the gastric juice after histamine injection. There are no neurological symptoms. These cases are easily differentiated from cases of the chlorosis type of which we recently observed some instances.

---

[1] Johnson, R., and Berglund, H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 517.

[1] Wu, Hsien, and Wu, D. Y., *Chin. J. Physiol. Report*, Ser. No. 1, 1928 (July), 135.

While these factors were becoming clear in our minds, 2 fresh instances of anemia of this type came under observation. The patients were young soldiers, for a long time living under severe deprivation and lately for 2 months suffering from diarrhea of unknown origin. The first patient (Chart 1) was placed on the Hospital's ordinary Chinese diet. This diet is a well balanced diet, meat free and predominantly vegetarian, but decidedly better in quality than the ordinary diet of the poorer classes. After an observation period cod liver oil was given in doses of 15 cc. daily. The dramatic effect on the reticulocytes, in the form of an increase, from about 1% to 30% on the fourth day of the cod liver oil medication is brought out in the chart, as well as the general behavior of the blood during the

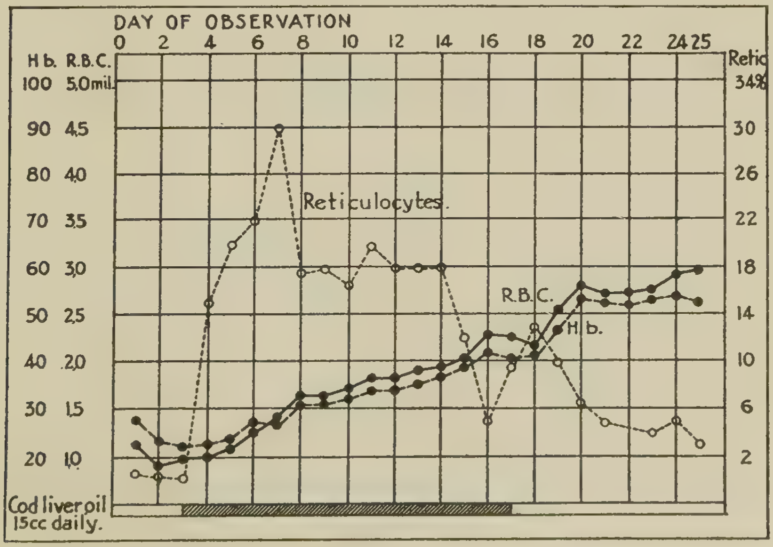


CHART 1.

first 25 days of observation. The blood continued to improve all way back to normal.

The second patient was for 3 days given cod liver oil, which had first been boiled for 10 minutes. Chart 2 shows the reticulocyte response which followed, as well as the general improvement of the blood. On the 19th day ordinary cod liver oil was given and after some delay a second but lower peak occurred on the reticulocyte curve. It will be noticed that the reticulocyte increase associated with the brief administration of the boiled cod liver oil was less marked than in the first case.

Chart 3 shows the similar response to cod liver oil in a milder case of anemia associated with pregnancy (I—para, age 22, cod liver oil started 14 days after delivery. This patient had earlier been treated with liver diet with no effect). As usually when the treatment starts at a higher erythrocyte level the reticulocyte response is less marked. The recticulum in each reticulated cell in all our observations has been surprisingly abundant.

Simultaneous with the observations presented in Charts 1-3, controls with cod liver oil were run in other anemias (kala azar, splenomegaly, and splenectomized cases, as well as cases of the chlorosis type) without any effect on the reticulocytes or on the anemia.

The anemias of the chlorosis type recovered on iron medication but without any increase in the reticulocytes.

We interpret the secondary anemia just described as associated with a deficiency in a fat soluble food factor. Extra factors seem necessary to bring the anemia into existence. As such factors we have observed prolonged diarrhea and pregnancy, both factors

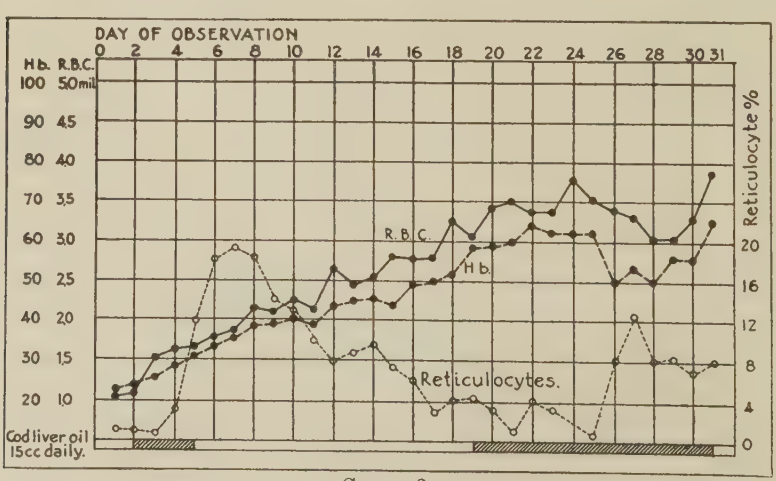


CHART 2.

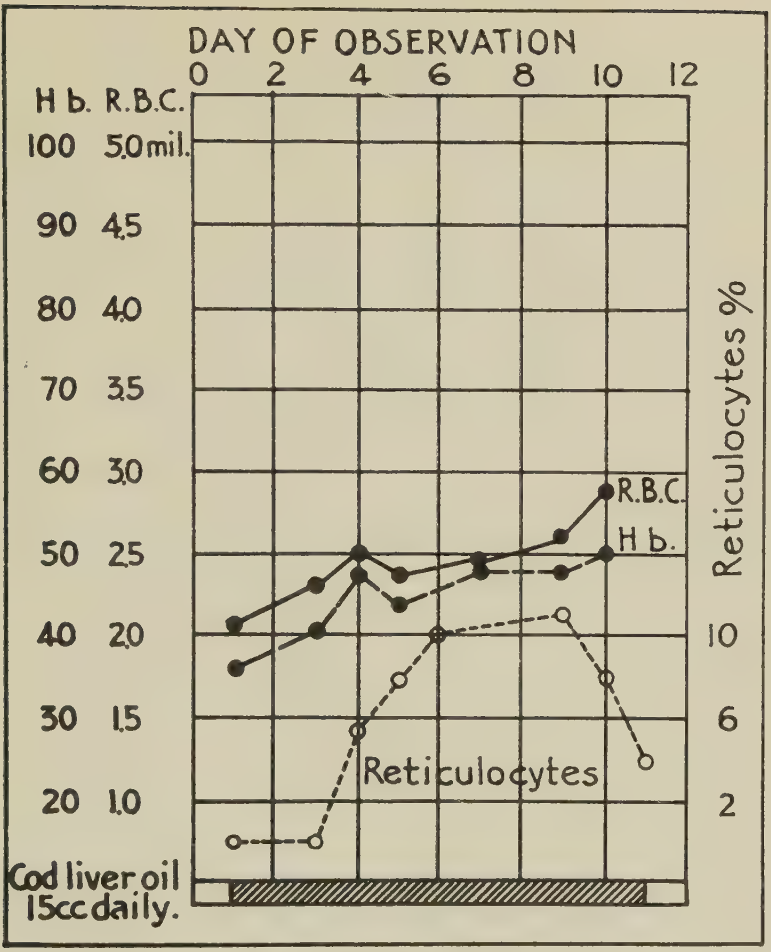


CHART 3.

known to be of importance in the field of hematology. The pronounced reticulocyte feature of this anemia so far seems to distinguish this new form from all other non-hemolytic secondary anemias known to occur in man.

4327

Transitory Character of the Achlorhydria During Fever Demonstrated by the Histamine Test.

HILDING BERGLUND AND H. CHIEN CHANG.

From the Department of Medicine, Peking Union Medical College, Peping, China.

The transitory achlorhydrias might present a clinical material suitable for the study of the unknown mechanism by which a strong mineral acid is being constantly produced by certain cells of the gastric mucosa. Whether this type of achylia will prove more useful than animal experiments depends upon several factors, one being the pathogenesis of this transient inhibition. Faber[1] has recently restated his well known view upon the achylias, including the transitory forms, as expressions of an anatomically manifested gastritis. Faber mentions the occurrence of achylia in intestinal diseases, particularly dysentery, typhoid, and paratyphoid fever, and points out the slow and gradual recovery from the achylia as the common course of events. Observations during induced fever in dogs and during experimentally produced hyperthermia led Carlson[2] and his co-workers to reckon with functional inhibition of cell activity rather than with histological changes of more stable nature.

By substituting the histamine test for the test meal the gastric secretion can be studied during severe fevers without inconvenience to the patients. In this series the histamine dose has been 0.5 mg. ergamine (Burroughs Welcome). The test has been carried out on a fasting stomach. The stomach was not washed previous to the subcutaneous injection, but the fasting contents had been withdrawn. With this small dose of histamine the highest free acidity was obtained in the juice secreted during the second 10-minute period after the injection. The sample analyzed represents the secretion during the first 20-minute period after the injection. Thus the values are not strictly maximum values. Günzburg's test has been carried out on all samples giving a free acidity below 20. We consider this essential.

Under the conditions described achlorhydria has been found in 23 patients with fever varying between 38.4° C. and 40.4° C. Of the 23 patients 9 had typhoid fever, 2 paratyphoid infection, 4 tuberculosis, 1 bronchopneumonia, 1 lung abscess and 1 cirrhosis of the liver. The causative relationship between the fever and the achlorhydria is supported in different ways. First, achlorhydria as an incidental finding is very rare in Peking, as demonstrated by the fact that only one such case of achlorhydria (28 year old patient with urticaria) has been found among 284 consecutive histamine tests carried out in our Out-patient Department and wards during the last four months. Second, when contrasting our fever cases against a series of cases of intestinal disease without fever free hydrochloric acid was regularly found in the latter. Thus 6 cases of acute bacillary dysentery all showed free HCl varying between 20 and 77 cc. 0.1 N acid per 100 cc. Likewise 8 cases of chronic dysentery (1 amoebic and 7 bacillary) all showed free acid, varying between 14 and 74. One of these cases had a duration of 17 months. Third, 10 of our cases with achylia during fever, were reexamined after the fever had subsided (Table I). Eight out of these 10 cases showed a return of free hydrochloric acid in a concentration between 22 and 106 cc. 0.1 N acid per 100 cc. The second test, showing the return of free acid, was carried out a short time after the temperature had become normal, the number of days of normal temperature varying between 4 and 19. In 2 cases of typhoid fever free HCl had not returned on the 8th and 25th day respectively of normal temperature. In all cases where the free acid recurred there was an increased amount of secretion after the fever, as compared with the fever period.

---

[1] Faber, Knud, *Lectures on Internal Medicine*, New York, Hoeber,1927.

[2] Meyer, J., Cohen, S. J., and Carlson, A. J., *Arch. Int. Med.*, 1918, xxi, 354.

TABLE I.

Showing time and extent of the return of the hydrochloric acid secretion after acute fever disease. Acidity expressed in cc. 0.1 N NaOH per 100 cc. Indicators: Dimethylaminoazobenzene and phenolphthalein. Sample withdrawn 20 minutes after histamine injection (0.5 mg. Ergamine, Burroughs Welcome).

Age	Diagnosis	Gastric analysis during fever					Gastric analysis during convalescence			
		Day of disease	Temp. C.	Vol. cc.	Free HCl	Total acidity	Day of norm. temp.	Vol. cc.	Free HCl	Total acidity
18	Typhoid	23	39.0		0	32	19	30	72	77
30	Resp. Inf.				0	3		14	22	40
24	Typhoid	13	38.9	9	8*	45	9	34	78	90
28	Typhoid	11	38.9	25	1*	9	10	83	62	71
24	Paratyphoid	10	38.8	35	11*	18	7	53	48	56
19	Typhoid	21	39.5	27	4*	20	5	58	106	111
17	Typhoid	15	39.0	12	0	14	12	23	30	38
22	Typhus	16	39.6	16	0	10	4	25	52	59
23	Typhoid	26	38.9	14	0	16	8	11	10*	18
34	Typhoid	60	39.0	15	0	7	27	7	6*	28

*Günzburg negative.

Minnesota Branch.

University of Minnesota Medical School, February 27, 1929.

4328

Cell Types and Their Proportion in Pars Anterior of Adult Male Human Hypophysis.*

A. T. RASMUSSEN.

From the Department of Anatomy, Medical School, University of Minnesota, Minneapolis.

There is considerable disagreement on the number of distinct cell types in the anterior lobe of the hypophysis, as well as on the question of the relative number of each type under normal conditions. As many as 7 different kinds of epithelial cells have been described. How many of these are merely different functional stages of the same cell is not settled; but for practical purposes, at least 3 kinds can be regularly recognized by relatively simple technique. They are: chromophobe (chief, reserve, clear, undifferentiated, neutrophil), acidophil (eosinophil, oxyphil, alpha) and basophil (cyanophil, beta).

The tendency seems to be to regard the acidophils as predominating and either the chromophobes or basophils as the fewest in number, although a few authors estimate that the chromophobes are the most numerous. An increase in the relative number of basophils in old age is also a widespread notion.

It is obviously of prime importance to settle these questions in order to properly interpret abnormalities in the hypophysis, such as are now being reported frequently in the pathological literature.

This is a preliminary report on a differential count of the cells in the anterior lobe of 100 human hypophyses from normal male adults, according to the method outlined in a previous communication.[1] The only change introduced since then is to put the section

---

* Aided by a grant from the Medical Research Fund of the Graduate School, University of Minnesota.

[1] Rasmussen, A. T., and Herrick, Ruth, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 416.

first for about one-half minute in ordinary hematoxylin and washing well with distilled water before staining with Mallory's connective-tissue stain.

Only cases of sudden and usually accidental death, in which the hypophysis was fixed within 12 hours after death, were used. They ranged in age from 18 to 78 years.

The chromophobes represent on an average 52% of the total cells (from 34% to 66%). The scarcity of chromophobes, so often mentioned, is not substantiated, since they are normally never below one-third of all the cells. They are also the least variable, the coefficient of variation being 15. Many of these cells contain very little cytoplasm so that they do not appear as numerous as an actual count indicates.

Acidophils are next in number, averaging 37% (23% to 59%). The coefficient of variation is 21. In only 5 cases did they constitute one-half or more of the total number of cells. The reason why so many authors consider the acidophils to be the most numerous is very likely that they are very conspicuous, because of their size and staining reaction. In hematoxylin and eosin specimens there is also the danger of including some of the chromophobes. Since larger cells are cut into more slices than the smaller ones, only cells with nuclei should be considered and this is not generally done in rough estimations.

Basophils are the least numerous. They represent only 11% of the cells. They are also the most variable (4.5% to 27%). The coefficient of variation is 34.

Only the approximate body weight is available so that no strict correlation between the percentage of the different cells and body weight is possible. However, 14 cases (several of them conforming to the general external features of the so-called non-neoplastic post-adolescent hypopituitarism) of excessive weight (200 to 330 lbs.) were compared with the remaining 86 with body weights less than 200 lbs. Statistically the difference is not significant except possibly in a decrease of 4.6% in the acidophils in the heavier group.

There is no significant correlation between stature and the percentage of different cells. From the association of growth with the acidophils, one might expect more acidophils in taller individuals. The anterior lobe as a whole is larger in taller people;[2] but evidently this is not due to an increase in any one type of cell.

When the percentage of the different cells of those below 50 years of age are compared with those above 50 years, there is an increase of 3.7% in chromophobes and a decrease of 3.9% in acidophils in the older group; but the supposed increase of basophils with advancing years is not corroborated. The coefficient of correlation *(r)* between age and percentage of basophils is —.022± .065.

---

[2] Rasmussen, A. T., *Amer. J. Anat.*, 1928, xlii, 1.

4329

Experimental Tularemia in Muskrats.

R. G. GREEN, E. M. WADE AND E. T. DEWEY.

From the Cooperative Investigation of the University of Minnesota and the Bureau of Biological Survey.

As one phase of a study of disease in wild life, various animals and birds have been tested for susceptibility to tularemia. Included in the group tested is the muskrat. The muskrats utilized in this work were caught in the wild and kept in captivity for a period longer than 6 months. They were under observation in the laboratory for a period of 2 months previous to the inoculation and appeared to thrive.

Muskrat 1. Inoculated subcutaneously 4-20-28, with spleen of guinea pig dying the previous day from tularemia. Symptoms of illness were present 2 days later. The muskrat died on the fourth day. Necropsy showed enlargement of left inguinal nodes and marked infiltration at the site of inoculation. The liver was studded with fine nodules. The spleen did not show studding. There was no exudate in the pleural or peritoneal cavities.

Guinea pig 1. Inoculated with spleen of muskrat; died on the third day with both inguinal nodes enlarged; spleen and omentum full of nodules.

Guinea pig 2. Inoculated with liver of muskrat; died on the third day with both inguinal nodes enlarged; intensive infiltration over abdomen. Spleen and liver were thickly studded with nodules.

Control guinea pig. Died on the fourth day with findings typical of tularemia.

Muskrat 2. Inoculated subcutaneously 4-20-28, with infective guinea pig spleen. Developed symptoms of illness 2 days later and died on the fourth day. Necropsy showed enlargement of left inguinal nodes and infiltration at the site of inoculation. Spleen was very large and thickly studded with nodules. Pleural and peritoneal exudates were present.

Guinea pig 1. Inoculated with liver from muskrat; died on the fourth day with enlarged lymph nodes. Spleen and liver thickly studded with nodules.

Guinea pig 2. Inoculated with peritoneal fluid of muskrat; died on the fourth day, showing enlarged inguinal nodes, with spleen and liver thickly studded with nodules.

Muskrat 3. Inoculated 5-29-28, subcutaneously, with spleen of guinea pig dying from tularemia; died on the sixth day. Necropsy showed inguinal nodes on both sides enlarged. Liver and spleen were studded with typical nodules of tularemia.

Muskrat 4. Inoculated 5-29-28 with spleen of guinea pig, dead from tularemia. Inoculation was made by scarifying the skin and rubbing an emulsion of the infective tissue into the abrasion. Died on the sixth day, showing inguinal nodes on both sides very much enlarged. Spleen and liver were thickly studded with nodules which were confluent to such an extent that there were large areas of necrosis in the liver.

Muskrat 5. Inoculated 5-29-28, by rubbing spleen of guinea pig into an abrasion of the skin. Died on the sixth day. Inguinal nodes enlarged; spleen and liver thickly studded with nodules.

It appears that the muskrat is very susceptible to an experimental infection with *B. tularense*. The pathology produced by the infection is similar to that seen in rabbits and guinea pigs, but the lesions in the muskrat are apt to be more marked. It is indicated that tularemia may occur in nature as a disease of muskrats.

4330

Fundamental Food Requirements for Growth of Rat. V. Influence of Fat in Diet.*

L. S. PALMER AND CORNELIA KENNEDY.

From the Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul.

Evans and Burr[1] have postulated the existence of a new vitamin, (F), revealed in part by subnormal growth of rats fed synthetic diets extremely low in fat, and containing sucrose as the source of carbohydrate. Although the first report of their work indicates that the casein and sucrose employed as sources of protein and energy require special purification in order to demonstrate the existence of the new vitamin, later, more detailed, reports show that little improvement is secured with commercial casein and sucrose. However, great improvement in growth is noted when fat is included in the diet or when liver or lettuce is fed in addition to the usual supplements of yeast and cod liver oil. Evans and Lepkovsky[2] have offered a partial explanation of these results in the hypothesis of a sparing action of fat on the antineuritic vitamin requirement for growth, but this view does not explain the beneficial effects of the lettuce and liver.

---

* Published with the approval of the Director, as Paper No. 836, Journal Series, Minnesota Agricultural Experiment Station.

[1] Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 740; *ibid.*, 1928, xxv, 390.

We present here the results of several experiments with rats fed diets containing various proportions of fat without lettuce or liver supplements. Data are given for both sexes. The casein was in all cases the highly purified product described elsewhere.[3] Commercial sucrose was used in the sugar diets. The dextrin was tapioca autoclaved at 260° F. for at least 6 hours. All the fat diets contained at least 9% butterfat, the remainder of the fat being crisco or lard. Salt mixture, either McCollum's No. 185 or Osborne and Mendel's, comprised 4% of the diets. Except for Groups No. 4 and No. 9 each rat received separately each day pure dry yeast (Northwestern Yeast Company) and cod liver oil. The daily quantity of yeast was 600 mg. for Groups Nos. 2, 3, 5, 6, 8, and 10, 400 mg. for Group No. 9, 250 mg. for Group No. 1. The quantity of cod liver oil fed was 160 mg. for Groups Nos. 2, 3, 5, 6, 8, and 10, and 500 mg. for Group No. 9. In Group No. 1 the fat-free portion of the diet was irradiated for 30 minutes with a Hanovia Alpine Sun Lamp. Groups Nos. 4 and 9 received no special source of vitamin D, and the antineuritic and antipellagrous vitamins were supplied by alcoholic extract of wheat embryo, equivalent to 30% of the ether-extracted material in the diet.

All the rats were on screens (3 mesh per inch) except Groups Nos. 3 and 6.

For comparison our results are reported in the same tabular form used by Evans and Burr. We also report the additional data of average food consumption (dry matter) per rat between the 28th and 90th days of life, when the rats were on the experimental diets, as well as the efficiency with which the food consumed was used for gain in weight for the first 6 weeks of the experimental period. Evans and Burr do not report any food consumptions except for the special supplements.

---

[2] Evans, H. M., and Lepkovsky, S., *Science*, 1928, lxviii, 298.

[3] Palmer, L. S., and Kennedy, C., *J. Biol. Chem.*, 1927, lxxiv, 597.

Our experiments show no relationship between the growth attained and the fat content of the diet. In this respect they confirm the early findings of Osborne and Mendel[4] and Drummond and Coward,[5] who obtained normal growth of rats on diets extremely poor in or "devoid of true fats." The best growth for both sexes was secured with the fat-free-sugar diets which are supposed by

TABLE I.

Growth and food utilization on diets containing various proportions of fat.

Group No.	Characteristics of Rations			Sex	No. of rats	Range in final wt.	Mean wt. at 90 days of age	Dry matter eaten during exp. per rat	Efficiency of food utilization*
	Fat	Protein	Kind of Carbohydrate						
	%	%				gm.	gm.	gm.	
1	24	35	dextrin	♂	6	170-240	210	512	1.50
2	0	25	sucrose	♂	4	228-238	232	635	2.79
3	0	25	sucrose	♂	2	254-266	262	704	2.34
Evans and Burr	0	25	sucrose	♂	—	—	100	—	—
4	15	18	dextrin	♀	6	140-176	152	430	5.16
5	0	25	sucrose	♀	2	150-186	168	508	4.23
6	0	25	sucrose	♀	2	158-180	169	477	3.44
7	24	35	dextrin	♀	6	154-184	173	470	3.10
8	9	25	sucrose	♀	4	176-194	185	534	3.92
9	15	18	sucrose	♀	6	174-226	194	583	3.64
10	0	25	sucrose	♀	2	206-212	209	576	2.54
Evans and Burr	0	25	sucrose	♀	21	108-162	138	—	—
Evans and Burr	5-22	25	sucrose	♀	51	140-220	171	—	—

*Food (dry matter) consumed per gm. gain per 100 gm. body weight for the first 6 weeks of the experimental feeding, beginning at 60 gm. body weight; (food ÷ gain) ÷ mean wt. during experiment.

---

[4] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1920, xlv, 145.

[5] Drummond, J. C., and Coward, K. H., *The Lancet*, 1921, ii, 698.

Evans and Burr to depress the growth rate. All of our rats that had not attained their normal mature weight were still growing at a regular rate at 90 days of age. In addition, none of them exhibited any visible external bodily defects or lesions. They would have been judged as normal in every way by the most critical observer. All of our female rats on the fat-free-sugar diets were as heavy as the average of Evans' and Burr's rats on their fat-containing diets. Most of them were heavier. This comparison is valid inasmuch as the mean mature weight of the females of our stock is the same as that of Evans and Burr.

Among the male rats a comparison of Group No. 2 (screens) and Group No. 3 (sawdust) indicates some advantage of free access to feces. However, among the female rats Group No. 6 (sawdust) was no better than Group No. 5 (screens).

The food consumption and food utilization data give the explanation of the differences in growth among the different groups. For both males and females the increasingly greater growth rate of the groups as arranged in the table is associated with either increased food consumption or greater efficiency of food utilization or by both of these factors. There is no evidence that the presence of fat in the diet contributed in any way to the changes in food intake or its utilization for growth.

It seems to us that there are no convincing data at hand to warrant the postulation of an essential growth vitamin F associated with fat. We venture the opinion that the differences observed by Evans and Burr could be readily explained on the same bases as our own, if the data were available.

4331

Induction of Menstruation in Women by Use of Ovarian Hormone.*

J. F. MC CLENDON, GEORGE BURR AND F. F. WILDEBUSH.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

In cooperation with Claire Conklin, it was found that the basal metabolism of half the women students examined was low and was about 15% below the Aub-Dubois standard. Some of these were studied for prolonged periods but others for only one day so that, in the latter, variations were not considered. It is a significant fact that all of the cases of amenorrhea fell in the low basal group. The basal metabolism of the following women was at times 15% below normal but showed slight variations.

---

* This research was aided by grants from the Therapeutic Research Committee, American Medical Association, and from the American Association for the Advancement of Science.

In a previous communication we reported the use of injections of 2000 to 4000 mouse units of ovarian hormone in women and that it was excreted in the urine. Owing to this fact we supposed that repeated injections would be necessary in order to have any marked effects. Consequently, we tried the effects of daily injections into a woman, 19 years old, who had not menstruated for 5 months. One thousand mouse units were injected every day for 9 days. Then they were stopped. At the end of 3 weeks she had a menstruation which lasted 6 days. A second woman, 22 years old, who had amenorrhea for 5 years and had not menstruated at all for 6 months, was given 1000 mouse units a day for 9 days. The injections were stopped for 2 weeks and then began again, when 1000 mouse units were injected every day for 6 days and then stopped again. Five days later she began to menstruate and menstruation lasted 2 days. The fact that menstruation occurred after the second series of injections and not after the first may be due to the possibility that enough hormone had not been injected in the first series. Although there are only 2 cases reported, menstruation occurred in 100% of the cases that we tried on women who possessed ovaries and who had menstruated sometime during their lives. Some other experiments indicate that larger or more frequent doses may be necessary in case the patient's ovaries are not producing some of the hormone. Most of the hormone used was obtained from Parke, Davis & Company and part of it from Squibb and Sons. Before injection we extracted it with peroxide-free ether and evaporated the ether to a small volume and mixed it with Ringer's fluid, boiling off the ether.

Western New York Branch.

Clifton Springs Sanitarium, February 23, 1929.

4332

Fate of Glucose and Other Sugars in the Eviscerated Animal.

CARL F. CORI AND GERTY T. CORI.

From the State Institute for the Study of Malignant Disease, Buffalo.

Rats fasted previously for 24 hours were operated upon under amytal anesthesia. The whole intestinal tract, including spleen and pancreas, and occasionally kidneys and adrenals, was removed. The blood sugar falls in such a preparation owing to the fact that the liver is out of circulation. The sugar content of the whole rat was determined by passing the heat coagulated tissues through a meat grinder and extracting them with hot water. Separation of sugar from non-sugar reducing substances was attempted.

Sugar (100 mg. per 100 gm. rat) was injected intravenously after evisceration and groups of rats were killed and analyzed, (a) 10 minutes after the sugar injection, (b) after 1 hour of sugar injection at a constant rate, and (c) after 1 hour of sugar injection plus insulin. The average percentages of sugar recovered in the 3 cases are shown in the following summary:

	10 minutes	1 hour	1 hour (insulin)
Glucose	97	90	2
Mannose	91	60	54
Fructose	98	61	62
Dihydroxyacetone	80(92)*	67	63

* 1 minute after injection.

The specificity of the insulin action is a very striking phenomenon. In experiments with injection of larger amounts of sugar and extending over 2 hours, glucose, in the absence of insulin injection, was found to disappear to the same extent as the other sugars. The glycogen content of the muscles increased under these conditions.

4333

On the Mechanism of Glucose Absorption from the Intestinal Tract.

CARL F. CORI, GERTY T. CORI AND HILDA L. GOLTZ.

From the State Institute for the Study of Malignant Disease, Buffalo.

Pierce and collaborators[1] recently published 2 statements: (1) The rate of absorption of glucose falls off during the last hour of a 3 hour period. This, they say, does not agree with Cori's findings. (2) The percentage of glucose absorbed during 1, 2 and 3 hour periods depends upon the amount of glucose remaining unabsorbed in the alimentary tract. The experimental data on which these 2 statements rest, appeared simultaneously elsewhere.[2]

The decrease in the rate of absorption in the third hour is not difficult to explain, because Pierce did not feed enough sugar to let the absorption proceed for 3 hours. Whereas an average of 236 mg. of sugar was absorbed in the first hour and of 229 mg. in the second hour, only 88 mg. of sugar were available for absorption in the third hour. Pierce erroneously assumed that absorption was not completed after 3 hours, because he made no allowance for the blank (sugar plus non-sugar reducing substances) given by empty intestines. In our experiments[3] an average of 235 mg. of glucose remained unabsorbed in the intestine after 3 hours. In this case the blank can be neglected, because it constitutes only a small percentage of the sugar content of the intestine, especially if the determinations are made with the less sensitive Bertrand method. In the experiments of Pierce there remained an average of only 44 mg. of sugar per 100 gm. rat. In 4 individual experiments 24, 10, 24, and 18 mg. of sugar per 100 gm. rat were found remaining in the intestine. This corresponds to an absorption of 95.5 to 97.7% of the sugar fed, which is evidently complete absorption, considering that Pierce used the Folin-Wu method* and made no allowance for the rather high blank which this method gives for intestines of fasting rats.

The fact that Pierce and collaborators found a constant rate of absorption in the first 2 hours, is a confirmation of our results. It was reasonable to expect that the mechanism of absorption would remain the same for all hours and not suddenly change after the second hour. Disregarding his own results, Pierce tries to show by means of a calculation that the amount absorbed is a function of the sugar remaining in the intestine. At the same time, he states that he observed no relationship between the amount of sugar fed and absorbed. Obviously, these 2 statements are contradictory and only one can be true.

---

[1] Pierce, H. B., Osgood, H. S., and Polansky, J. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 347.

[2] Pierce, H. B., Osgood, H. S., and Polansky, J. B., *J. Nutrit.*, 1929, i, 247.

[3] Cori, C. F., *J. Biol. Chem.*, 1925, lxvi, 691.

* Personal communication.

The gist of Pierce's calculation is that it is merely a coincidence that Cori observed a constant rate of absorption, because he fed increasing amounts of glucose in the 1, 2 and 3 hour absorption periods, compensating thereby for a decrease in the rate of absorption which otherwise would have occurred in the second and third hour. There is evidence that coincidence plays no rôle in the finding of a constant rate of absorption but is concerned in the rough agreement of Pierce's calculation with the results obtained in one particular set of experiments. Since Pierce repeated these experiments, he also found agreement with his calculation. However, if he had calculated some of our other experiments in which no progressive amount of sugar was fed, he would have noted that the observed and calculated values do not agree at all. This is also shown in the following new experiments in which a constant amount of glucose was fed for each of the absorption periods. (Table I.)

TABLE I.

Four rats each killed 1, 2 and 3 hours after glucose feeding. The average amounts fed per 100 gm. rat were 943 mg. for the 1 hour, 931 mg. for the 2 hour and 951 mg. for the 3 hour absorption period. The average body weight of the 3 groups of rats was 140.5, 142.6 and 138.2 gm. respectively.

Glucose absorbed per 100 gm. rat.

0 to 1 hour		0 to 2 hours		0 to 3 hours	
mg.	%	mg.	%	mg.	%
200	17.7	470	45.2	666	66.7
232	24.7	438	51.1	693	75.3
166	19.0	458	51.2	690	76.6
214	25.5	510	54.6	594	65.0
203	21.7	469	50.5	660	70.9
Expected according to Pierce*		363	38.6	489	51.9

*Calculation: Since 21.7% of 943 is absorbed in the first hour, 740 mg. of sugar are left in the intestine after one hour. 21.7% of 740 is supposed to be absorbed in the second hour, leaving 578 mg. for the third hour and so on.

The results in Table I are those usually obtained when young and healthy male rats of uniform age and weight are used. Pierce departed in this particular from the method as originally described, since he used less uniform and much heavier rats.

The experiments in Table I show that the rate of absorption is not a function of the amount of sugar present in the intestine. If such were the case, the observed and calculated values would agree. Also, there is no falling off in the rate of absorption in the third hour even though only 29% of the sugar fed remains in the intestine unabsorbed. Previous experiments showed that absorption can proceed still further or can even go to completion without leading to a noticeable decrease in the rate of absorption. This is strikingly illustrated in new experiments which were carried out to emphasize this point again. (Table II.)

TABLE II.
Killed 3 hours after glucose feeding.

Body weight	Per 100 gm. of body weight			% absorbed
	Glucose fed	Glucose remaining in intestine	Glucose absorbed	
gm.	mg.	mg.	mg.	
138.2	706	14.3	692	98.0
110.5	719	28.2	691	96.1
125.0	730	58.0	672	92.1
121.7	712	29.6	682	95.8
	717	33.0	684	95.5
Average of Table I			660	

The highest absorption in the 3 hour period in Table I was 693 mg. per 100 mg. rat. Slightly more than this amount (717 mg.) was fed to 4 rats and the sugar remaining in the intestine after 3 hours was determined.* In this way just enough sugar is introduced to permit the absorption to proceed for 3 hours and consequently the sugar in the intestine falls to a low value toward the end of the 3 hour absorption period. Since the absorption proceeds at an average rate of 660/3 = 220 mg. per hour (Table I), 100 mg. or 14% of the glucose fed would be left in the intestine after 150 minutes. If this small amount of residual sugar were a limiting factor, the rate of absorption would diminish in the last half hour, and consequently more sugar would remain in the intestine than was found experimentally (Table II).

Perhaps no better example can be offered for the fact that the rate of absorption of glucose is uninfluenced, within widest limits, by the amount of sugar present in the intestine than the experiments in Table II. It may be mentioned that other substances are not absorbed in the same way as sugars. For instance, the rate of absorption of sodium lactate depends on the amount fed.[4] The mechanism of absorption of other substances will soon be reported.

---

* The intestinal washings were precipitated with colloidal iron and made up to 200 cc. An aliquot portion of the filtrate was shaken out with Lloyd's reagent and the reducing value was determined by means of the Hagedorn and Jensen method. The Lloyd filtrate was treated with copper sulfate and lime in order to remove sugar and the reducing value again determined. The difference represents sugar in the sample. In the experiments in Table I, where more sugar was left in the intestine, Bertrand's method was used.[3]

4334

Influence of Food Fat on the Constitution of the Phospholipids.

ROBERT GORDON SINCLAIR. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York.

In the course of an investigation concerning the mechanism of fat absorption, it was thought that possibly the phospholipids of the intestinal mucosa are intermediary products in the process of fat resynthesis from the absorbed fatty acids and glycerol. If this be true, during the absorption of fat the constituent fatty acids of the phospholipids in the mucosa must consist, in part at least, of the fatty acids of the ingested fat. In order to test this idea it was decided to see if the absorption of such fats as cod liver oil and cocoanut oil (which are characterized by high and low degrees of unsaturation, respectively) produces a change in the iodine absorption value (I.N.) of the phospholipid fatty acids in the absorbing mucosa. Later the investigation was extended to include a study of the effect of fat absorption and of different continued diets on the constitution of the phospholipids of the intestinal and skeletal muscles and of the liver.

The experimental plan was as follows: To one of a pair of cats which had been kept on the same diet for 2 weeks or longer either cod liver oil or cocoanut oil was administered by stomach tube; some time later (usually 5-7 hours) the animal was killed. The other cat was killed in a post-absorptive state to serve as a control. The mucosa and the muscle of the small intestine were separated; the muscles of one hind leg were used to typify skeletal muscle. The lipids were extracted with hot alcohol and the phospholipids were isolated and purified by acetone precipitation from ether solution, according to the method of Bloor.[1] After saponification of the phospholipids, the weight and I.N. of the constituent fatty acids were determined.

---

[4] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1929, lxxxi, 389.

[1] Bloor, W. R., *J. Biol. Chem.*, 1926, lxviii, 33.

TABLE I.

Fatty Acids of the Acetone-Insoluble Lipids.

Tissue	Preliminary diet	Controls; fasted 18 to 24 hrs.			Fasted 18 to 24 hrs.; then fed oil.				
		Weight in tissue		Iodine number	Iodine number	Weight in tissue		Oil fed	Time of feeding before death
		moist	dry			moist	dry		
		%	%			%	%		hours
Mucosa of small intestine	beef kidney	1.03	6.35	112±1.6	103±4.1	0.92	5.60	C. O.*	6
	meat scraps	1.13	7.44	94±2.1	107±1.5	1.15	7.21	C. L. O.	5 to 7
Muscle of small intestine	beef kidney meat scraps lean beef and bread	0.43 0.59 0.70	2.12 3.06 3.83	108±2.9 97±1.4 92±1.5	104±4.5 99±0.6 93±1.5	0.39 0.64 0.60	1.81 3.37 3.26	C. O. C. L. O. C. L. O.	6 5 to 7 2 to 75
Skeletal muscle	lean beef and bread beef kidney	0.62 0.79	3.21 3.70	108±2.6 130±5.9	108±0.9 129±0.7	0.61 0.66	3.22 3.17	C. L. O. O. O.	2 to 8 10 to 12
Liver	lean beef and bread beef kidney	2.08 2.50	8.78 10.21	115±5.0 145±0.6	135±2.6 144±2.5	2.20 2.50	10.06 10.20	C. L. O. O. O.	4 to 8 10 to 12
Rat skeletal muscle	extracted casein, corn starch, salt and yeast	0.75	2.99	131±1.7	131±1.2	0.74	2.98	O. O.	4 to 96

*C. O. stands for Cocoanut Oil; C. L. O., for Cod Liver Oil; and O. O. for Olive Oil.

Results: The average values from several series of experiments (Table I) show: (1) There is no change in the amount of the phospholipid fatty acids in any of the tissues studied during the absorption of fat. (2) The fatty acids of the ingested fat are incorporated into the phospholipid of the intestinal mucosa and of the liver. (3) The composition of the phospholipid fatty acids in both smooth and skeletal muscle is not influenced by fat absorption. (4) The type of diet fed over a considerable period of time (2 weeks) has a significant influence on the degree of unsaturation of the constituent fatty acids of the phospholipids in the intestinal mucosa, the liver, and smooth and skeletal muscles.

These results would seem to indicate that the phospholipids have at least 2 functions: in the intestinal mucosa and in the liver part of the phospholipids fulfill a rôle in the absorption and assimilation of fat; in the muscles the phospholipids probably are vital constituents of protoplasm and, as has been suggested by Bloor,[2] undergo wear and tear and reconstruction.

Further work on the effect of diet and of starvation on the constitution of the phospholipids is in progress.

It is a pleasure to acknowledge the continued helpful advice received from Professor W. R. Bloor throughout this investigation.

4335

Comparison of the Icteric Index and the Direct Van den Bergh Tests.

ROGER S. HUBBARD AND CATHERINE B. ALLISON.

From the Laboratory of the Clifton Springs Sanitarium and Clinic, Clifton Springs, New York.

In 316 specimens submitted for the determination of the degree of pigmentation of the serum by the method of Meulengracht[1] the direct diazo reaction described by Van den Bergh[2] was also carried out. The table shows the relationship found between the results of the 2 tests. When the icteric index was high practically all specimens gave a positive diazo reaction, but when it was low the number of specimens which gave this result was much smaller. The time required for the reaction to become positive also varied with the

TABLE I.
Icteric Index and Direct Van den Bergh Tests Compared.

Index	Total	Tested	Neg.	Minutes for reaction to develop						
				0	5	10	15	20	25	30
units	No.	No.	%	%	%	%	%	%	%	%
to 7	68	25	56	0	0	0	16	20	24	32
7- 10	73	31	42	0	0	3	16	26	26	36
10- 12	72	72	18	1	1	8	18	40	51	71
12- 15	69	69	13	1	3	14	29	51	61	78
15- 20	45	45	16	9	9	20	36	64	71	80
20- 30	48	48	4	23	29	42	60	69	79	88
30- 50	12	12	0	58	67	75	100	—	—	—
50-100	2	2	0	100	—	—	—	—	—	—
over 100	12	12	0	100	—	—	—	—	—	—

---

[2] Bloor, W. R., *J. Biol. Chem.*, 1927, lxxii, 327.

[1] Meulengracht, E., *Deut. Arch. f. klin. Med.*, 1921, cxxxvii, 38.

[2] Van den Bergh, A. A. H., *Presse Médical*, 1921, xx, 441.

intensity of the yellow color (amount of bilirubin present). The time was decidedly shorter in the average specimen with a high index than when the index was low. The occurrence of an immediate reaction seemed in most instances to be a part of the expression of this decrease in the reaction time rather than of some independent phenomenon, such as a chemical difference in the nature of the bile pigment present. There were a few rather conspicuous exceptions to this orderly arrangement, and the authors believe that the presence of substances, other than bile pigments, which give the diazo reaction must be considered as a possible explanation for them.

4336

Tissue Respiration and Endocrine Function. III. Influence of Thyroidectomy on Tissue Respiration.*

J. A. DYE and G. H. MAUGHAN.

From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca.

Opinion differs in regard to the exact method by which the thyroid hormone exerts its influence on body metabolism. Plummer and Boothby,[1] Kendall[2] and others have accepted the view that its action is within the cells themselves and believe that it acts as a catalyst. Kunde[3] has shown that when daily repeated doses of thyroid substance are given to dogs a progressive increase in metabolism occurs as time advances, reaching a maximum in 3 weeks or more after the initial dose. This investigator states further that the height of response to a quantity of the thyroid substance depends upon some condition of the body cells, which become less and less resistant to repeated doses of the thyroid hormone, and that the catalytic theory of Plummer is inadequate to explain hyperthyroidism experimentally induced in dogs.

Data previously reported from this laboratory[4] would indicate that the apparent catalysis of the fundamental processes of metabolism induced by thyroid substance is, in part at least, indirect, *i. e.*,

---

* This work was aided by a grant from the Heckscher Research Foundation of Cornell University.

[1] Plummer, H. S., and Boothby, W. M., *J. Am. Med. Assn.*, 1924, lxxxiii, 1333.

[2] Kendall, E. C., *Endocrinology*, 1924, iii, 156.

[3] Kunde, M. M., *Am. J. Physiol.*, 1927, lxxxii, 195.

[4] Dye, J. A., and Waggener, R. A., *Am. J. Physiol.*, 1928, lxxxv, 1.

that it exerts its influence upon the body cells by increasing the amount, potency or some other property of the oxidation catalysts normally present. As measured by the indophenol oxidase activity, there is a diminution of from 10 to 50% in the respiratory power of the cells from tissues of cretin pups and lambs.

The present experiments were undertaken to determine the oxygen consumption of surviving tissues from cretin pups (thyroidectomized when 5 weeks of age, tissues studied 7 months later) as compared with their normal litter-mates of the same sex. If the diminished metabolic rate of the thyroidectomized animal is reflected in the surviving tissue, it follows that normally the thyroid hormone must accumulate in the cells, if its action is to be considered as catalytic in nature, or that it must affect, in some way, one or more of the normal constituents of the cells. Foster[5] reported a diminution of from 25 to 30% in the oxygen consumption of surviving strips of diaphragm muscle from thyroidless rats.

The oxygen consumption of finely minced skeletal muscle, in atmospheric oxygen, was measured by means of a differential volumeter, to be described later. The suspension fluid employed was a N/15 disodium phosphate solution in normal saline, which after adding the minced tissue (1 gm. to 3 cc.), now acid in reaction, gave a pH of 7.27. The water bath was kept at 39° C. ± 0.01. Representative readings for normal and experimental tissues from a single experiment are shown in the following table.

EXPERIMENT 11.

Oxygen consumption of surviving skeletal muscle of normal and thyroidectomized pups.

Animal	*M. biceps femoris* *M. semitendinous*						*M. adductor magnus* *M. semimembranous*					
	Time 10′	20′	30′	40′	50′	60′	10′	20′	30′	40′	50′	60′
Average cretin	107	209	306	389	451	526	94	186	271	345	411	462
Average control	155	305	462	610	726	825	155	308	443	550	638	710
Av. cretin % / Av. control	69.0	68.5	66.2	63.8	62.1	63.7	60.0	60.4	61.2	62.7	64.4	65.1

Substrate: 0.9 % NaCl; N/15 disodium phosphate. pH 7.27.
All volumes are reduced to 0° C., 760 mm.

These data, which we have readily duplicated in other experiments, show that for skeletal muscle of cretin pups, 7 months after thyroidectomy, there is a diminution of from 25 to 60% in their power to consume atmospheric oxygen; an average of from 30 to 40%. These low values would support the view that the thyroid hormone exerts an influence upon the oxidative systems of the cells. Further work is in progress.

---

[5] Foster, G. L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 334.

4337

Tissue Respiration and Endocrine Function. IV. Influence of Thyroidectomy on Succinoxidase Activity of Surviving Tissues.*

J. A. DYE AND G. H. MAUGHAN.

From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca.

The oxidation of succinic acid is a very suitable reaction for studying the oxidation capacity or respiration of a tissue, particularly muscle. Einbeck,[1] Moyle,[2] and others have shown that perfectly fresh muscle normally contains succinic acid; Thunberg,[3] Batelli and Stern,[4] *et al.*, have demonstrated in muscle and other tissues a very powerful enzyme for the oxidation of this acid; the activity of this enzyme is not diminished, in fact may be enhanced, by washing the tissues, a procedure which almost completely inhibits the action of other oxidizing systems either by its direct action upon the enzyme systems or by removing their normal substrates; and lastly, through the work of Thunberg, Einbeck and Fleisch[5] we are familiar with the details of the chemical changes occurring in this oxidative reaction. These investigators have shown that when succinic acid, $C_4H_6O_4$, is oxidized by the tissues it is transformed to fumaric acid, $C_4H_4O_4$, one atom of oxygen reacting with each molecule of the acid without the addition of water. Fleisch has further shown that the succinoxidase is probably composed of a dehydrogenase which is capable of transporting active hydrogen to any suitable acceptor, *e. g.*, active oxygen, methylene blue, etc., but before the former can function as a hydrogen acceptor it must first be activated by another enzyme. Warburg[6] believes that this depends

---

* This work was aided by a grant from the Heckscher Research Foundation of Cornell University.

[1] Einbeck, H., *Z. Physiol. Chem.*, 1913, lxxxvii, 145; *Ibid.*, 1914, xc, 301.

[2] Moyle, D. M., *Biochem. J.*, 1924, xviii, 351.

[3] Thunberg, T., *Skand. Arch. Physiol.*, 1909, xxii, 430.

[4] Batelli, F., and Stern, L., *Biochem. Z.*, 1911, xxx, 172.

[5] Fleisch, A., *Biochem. J.*, 1924, xviii, 298.

[6] Warburg, O., *Biochem. Z.*, 1921, cxix, 134; *Ibid.*, 1923, cxxxvi, 266.

upon the presence of iron. The oxygen activator only is inhibited by HCN.

The utility of this reaction lies especially in the fact that, through washing of the finely minced tissue, succinoxidase can be studied alone since succinic acid, in the form of its sodium salt, can be added to the substrate in optimum concentrations. Skeletal muscle from normal and cretin pups (thyroidectomized when 5 weeks of age, the tissues were studied 7 months later) was finely minced, forced through a fine sieve, washed with vigorous shaking for 8 minutes in 50 volumes of distilled water, filtered, rewashed twice more in the same manner, strained and pressed through fine-meshed gauze to remove as much water as possible, and their avidity for oxygen in the presence of succinic acid studied. In no case were the tests made sooner than 4 hours after the death of the animal.

The respiratory power of the tissues was measured by determining the volumes of oxygen consumed from atmospheric air, the oxygen serving as the hydrogen acceptor. For this purpose the differential volumeter used in the study of tissue respiration was employed. One-half gm. of the washed tissue was floated in 3 cc. of the substrate (N/30 disodium phosphate, 1% sodium succinate), the mixture having a pH of 7.45-7.5.

The data contained in the following table are a few representative readings from one experiment only. Similar values have been obtained in our other experiments, these will be reported in full at a later date. The oxygen uptake of washed muscle in the presence of succinic acid is more vigorous than that of unwashed surviving tissue. Without exception, the tissues from the cretin animals showed a diminution in their power to oxidize succinic acid. These

EXPERIMENT 11.

Oxygen consumption of washed skeletal muscle from normal and thyroidectomized pups, using succinic acid as a substrate.*

Animal	*M. Biceps femoris* *M. semitendinous.*				*M. adductor magnus.* *M. semimembraneous.*				
	Time 10′	20′	30′	40′	10′	20′	30′	40′	50′
Average Cretin	205	397	610	787	179	351	509	652	761
Average Control	244	482	683	875	217	436	630	786	925
$\frac{\text{Av. Cretin}}{\text{Av. Control}}\ \%$	84.0	82.4	89.3	90.0	82.5	80.5	80.7	82.0	82.6

*Substrate: 1% sodium succinate. N/30 disodium phosphate buffer. pH 7.4-7.5. All volumes are reduced 0° C., 760 mm.

vary from an average of 5% for pup 26, not a typical cretin, to 15% for pup 24 and 30% for pup 22. With all animals included, there is a diminution of 16% in the cretins.

These results offer additional support to the view that the thyroid hormone exerts an influence on the oxidizing systems normally present in the cells.

4338

Responses of the Kidney and Spleen to Subcutaneously Injected Epinephrin.

THEODORE KOPPANYI AND M. S. DOOLEY.

From the Department of Pharmacology of Syracuse University.

Halsey and Lemann[1] found that hypodermic injection of epinephrin in asthmatic patients may cause relaxation of the bronchial musculature without synchronous rise in blood pressure. This observation reopened the question of the differing sensitivity of various epinephrin receptors. C. A. Dragstedt and Huffman[2] recorded in dogs the simultaneous effects of continuous intravenous injections of epinephrin on blood pressure and intestinal motility during and after anesthesia and in the absence of drugs and anesthetics. Except under deep anesthesia, the authors were able to obtain definite blood pressure rises without intestinal inhibitions. Durant and McNinch[3] externalized the spleens of two young dogs by the Barcroft-Stephens method and took blood pressure and oncometric (spleen) tracings while the animals were unanesthetized. Intravenous injection of minimal amounts of epinephrin produced blood pressure rise and splenic constriction, but no intestinal inhibition.

Luckhardt and Koppányi[4] developed a method which greatly facilitated the attack of our problem. This method consists of subcutaneous injection of epinephrin and massage of the injected area. Such massages produced marked blood pressure rises, but, deepening the anesthesia promptly abolished the effects of massage upon blood pressure. When the effectiveness of massages of the epinephrinized areas upon the blood pressure is thus abolished, one can observe the action which such massages exert on different organs.

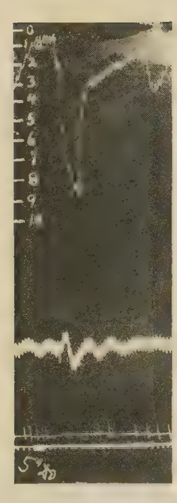
---

[1] Meyer and Gottlieb's Pharmacology, translated by Halsey. 346.

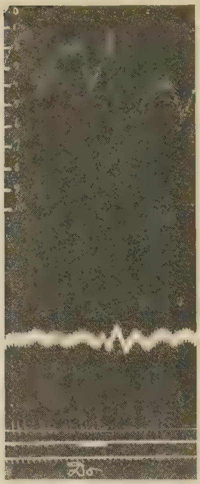
[2] Dragstedt, C. A., and Huffman, *Am. J. Physiol.*, lxxxv, 129.

[3] Durant and McNinch, *Am. J. Physiol.*, lxxxv, 364.

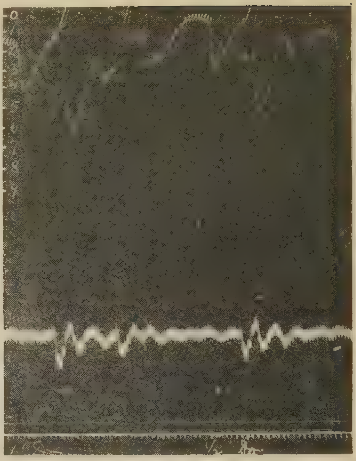
[4] Luckhardt and Koppányi, *Am. J. Physiol.*, lxxxi, 436.



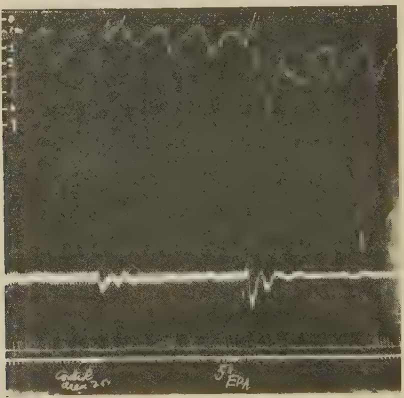
1



2



3



4

FIG. 1.

Simultaneous record of spleen volume changes (upper tracing) and of blood pressure (lower tracing). Vertical lines on the upper horizontal base represent drops of urine. "Do" equals massage of epinephrinized area for 5 seconds. Horizontal lines at the left margin indicate calibrations of volume changes in cc. of air. Base line (lower horizontal line) represents 0 mm. of Hg pressure. Time, 5 seconds.

FIG. 2.

Same arrangement as in Fig. 1. "Do" equals massage of epinephrinized area for 25 seconds. Time 5 seconds.

FIG. 3.

Same arrangement as in Fig. 1. "Do" equals massage of epinephrinized area for one and one and one half of one second. Time 5 seconds.

FIG. 4.

Same arrangement as in Fig. 1. The first experiment represents the massage of a control, the second of an epinephrinized area. Time 5 seconds.

This study deals with the effect of massage of epinephrinized areas on volume changes in the spleen and kidney. We know that intravenously injected epinephrin produces constriction of the spleen and kidney, and because of the latter checks the flow of urine. These actions are presumably due solely to vasoconstriction in these organs.

We used dogs for our experiments. The blood pressure was recorded from the common carotid artery, and the spleen or kidney was inclosed in an oncometer connected with a recording tambour. The ureters were cannulated and the urine flow registered by a drop recorder. The dogs were anesthetized by morphine-urethane reinforced with ether. Due to the depth of the anesthesia we obtained no, or only slight elevations of blood pressure upon massage of epinephrinized areas. 2 to 3 cc. of epinephrine hydrochloride were injected subcutaneously and the injected area was repeatedly massaged. As Figs. 1, 2, 3 and 4 show, the massages were for all practical purposes unsuccessful, so far as general blood pressure rises were concerned. But while the blood pressure showed no, or only slight (2-8 mm. of Hg.), rise, or even a fall (Fig. 4); the kidney and spleen manifested very marked constriction. Simultaneously, the urine flow stopped. Every massage of the injected area produced a marked volume reduction of the spleen and kidney, though, after oft repeated massage, the effect is slightly diminished. The volume changes of the spleen and kidney are undoubtedly due to stimulation of vasoconstrictor endings, but this vasoconstriction must be compensated for peripherally and thus it does not affect the general blood pressure. Massages of control areas produced no volume changes in the spleen and kidney.

These observations show:

(a) That massage of areas injected with epinephrin may produce marked localized vasoconstrictor effects, even when the general blood pressure remains unaffected.

(b) That kidney and spleen volume changes are more easily elicited with small amounts of epinephrin than a change in general systolic blood pressure. The volume changes of these organs may be used as a reliable criterion in the bio-assay of epinephrin.

Iowa Branch.

University of Iowa, February 7, 1929.

4339

The Thermal Increments and Critical Temperatures of Biological Reactions.

ELLIS I. FULMER AND R. E. BUCHANAN.

From the Departments of Chemistry and Bacteriology, Iowa State College.

It has become common practice to express temperature effects on rates of biological reactions in terms of the Van't Hoff-Arrhenius relationship by the use of one of the following equations:

$$1.\quad \text{Log}\, k = -\frac{\mu}{4.6T} + \text{constant.}$$

$$2.\quad \mu = \frac{4.6\,(\log k_2 - \log k_1)\ T_2T_1}{T_2 - T_1}$$

in which k_2 and k_1 are the velocity coefficients at absolute temperatures T_2 and T_1. The symbol μ has been called the thermal increment or thermal constant. The latter term is misleading since μ cannot be assumed to be a constant but its constancy must be proved for the temperature range under consideration. It is the purpose in this paper to discuss the value of μ as a function of temperature as calculated by means of Equation 2 for several biological processes and to point out instances where the use of Equation 1 has given misleading results.

In Table I are given values for μ calculated from data given by Sherwood and Fulmer[1] for the growth of *Saccharomyces cerevisiae* in various media. It is evident that μ decreases regularly with increase in temperature. In 3 of the media the value of μ is a linear function of the temperature. Moreover the medium used influences markedly the values of μ. It is of particular interest to note the influence of ammonium chloride both in the synthetic medium and in the beer wort. The notations μ_{10} and μ_5 have been used; since μ decreased regularly with increase in temperature its value will necessarily vary with the temperature range employed in its calculation.

---

[1] Sherwood, F. F., and Fulmer, E. I., *J. Phys. Chem.*, 1926, xxx, 738.

TABLE I.

Values of μ for yeast in various media. Sherwood and Fulmer.[1]

Medium	W_0		W_1		E_0		E_1		
T°	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	μ_5
0	1.22		4.53				1.13		
5	(2.6)		(7.4)				(2.55)		24,440
10	4.30	19,430	10.92	12,500	3.24		4.05	19,430	16,550
15	(7.1)	16,060	(14.0)	10,200	(5.2)		(6.20)	16,470	13,850
20	10.35	14,150	17.80	8,087	7.77	14,500	8.83	12,580	11,950
25	(13.5)	11,000	(19.4)	5,606	(10.4)	11,890	(11.20)	10,080	8,274
30	16.86	9,210	20.51	2,531	13.61	9,920	13.61	7,658	7,060
35	(19.0)	6,240	(20.4)	990	(13.4)	4,645	(14.2)	4,808	1,545
40	19.36	2,617	20.05	−1,403	13.16	−1,403	14.52	1,281	848

W_0 = beer wort without ammonium chloride. $\mu_{10} = 25,000 - 570t°$.

W_1 = beer wort with optimum concentration of ammonium chloride for each temperature. $\mu_{10} = 17,500 - 480t°$.

E = Synthetic medium optimum for 30°. μ_{10} is not a linear function of t°.

E_1 = Synthetic medium with optimum ammonium chloride for each temperature. $\mu_{10} = 26,000 - 620t°$.

An analysis of data by Slator[2] on the fermentation of different sugars by several yeasts shows that μ decreased regularly with increase in temperature. The value of μ for sporulation rate of *Saccharomyces pastorianus I* as calculated from the data of Herzog[3] is a linear function of the temperature, *i. e.*, $\mu = 44,100 - 1300\ t°$.

The above findings are not in harmony with the conclusions of Crozier[4] and Richards[5] for yeast. These authors calculated the value of μ by use of equation 1. They plotted log k against the reciprocal of the absolute temperature. Through these points straight lines were drawn, the slopes of which are $-\mu/4.6$. In Table II, I, are given values of μ calculated by means of equation 2 and compared to the values obtained by Crozier for the data of Slator[2] on the production of CO_2 by yeast at various temperatures. The data from the graph showed a "critical temperature" at about 21° while our figures show no critical temperature. This discrepancy is explained in the following manner. An examination of Eq. 2 shows that the value of μ, a large number, is determined by the accuracy of the difference between the logarithms of the velocity coefficients. In drawing a graph of log k against 1/T if the ordinate is not taken sufficiently large the differences in log k which are significant in showing the steady decrease in μ are masked. This is in effect decreasing the accuracy of the experimental values of the function. In the case in hand, the graphing of the values of log k with large enough ordinates shows plainly that log k is not through any temperature range a linear function of the reciprocal of the absolute temperature, but on the contrary decreases more rapidly than the variable.

---

[2] Slator, A., *J. Chem. Soc.*, 1906, lxxxix, 128; *J. Chem. Soc.*, 1908, xciii, 217; *J. Inst. of Br.*, 1911, xvii, 147.

[3] Herzog, R. O., *Z. physiol. Chem.*, 1902, xxxvii, 149.

[4] Crozier, W. J., *J. Gen. Physiol.*, 1924, vii, 189.

[5] Richards, O. W., *J. Phys. Chem.*, 1928, xxxii, 1865.

TABLE II.
Values of μ for several instances calculated by Eq. 2 and by Crozier[4] by means of Eq. 1.

Function	I			II			III		
t°	log k	μ(2)	μ(1)	log k	μ(2)	μ(1)	log k	μ(2)	μ(1)
4							0.718		16,700
5	0.1461		22,200						”
10	0.5378	28.320	”						”
14			”				1.263	19,940	”
15	0.8325	22,100	”						”
20	1.1139	21,840	”						”
24			12,250				1.744	18,870	”
25	1.1318	16,390	”	1.3665		16,700			”
30	1.4771	13,160	”	1.4949	10,660	”			”
34			”			”	1.959	9,020	”
35	1.6075	11,180	”			”			
40	1.7033	7,480	”	1.8416	15,120	”			
45				2.0177	16,150	”			
50				2.2518	22,120	”			

I — CO_2 production by yeast, Slator.[2]

II — Reduction of methylene blue by "resting" *B. coli*, Questel and Whetham.[6]

III — CO_2 production by heart ganglia of *Limulus polyphemus*, Garrey.[7]

In order to find whether a similar discrepancy occurred in other instances, values for μ were calculated by Eq. 2 and compared with those obtained by Crozier by the graphical method. Such values for the reduction of methylene blue by "resting" *B. coli* and for CO_2 production by heart ganglia of *Limulus polyphemus* show in the first case an increase of μ with rise in temperature and in the latter a decrease, as compared to a constant value of $\mu = 16{,}700$ for both cases as inferred from the graphical method.

It is of interest to note that the value of μ for malt amylase as calculated from data by Ernström[8] decreases regularly with rise in temperature, and for invectase according to Euler and Laurin[9] is a linear function of temperature, *i. e.*, $\mu = 22{,}800–205.2\ t°$. Spohr[10] found the value of μ for the inversion of sucrose by HCl in homogeneous system to be constant at 12,800 from 25° to 55°. This is in contrast to the reaction in heterogenous system. The fact that μ for single enzymes varies continuously with temperature would lend improbability to a constancy for a complex of enzymes such as are present in the living cell.

---

[6] Quastel, J. H., and Whetham, M. P., *Biochem. J.*, 1924, xviii, 519.

[7] Garrey, W. E., *Biochem. J.*, 1920, iii, 49.

[8] Ernström, E., *Z. physiol. Chem.*, 1922, cxx, 190.

It is concluded that μ varies continuously with the temperature in the several biological reactions examined. There is no evidence of a critical temperature nor of master or key reactions in a catenary series, as has been inferred by others from a supposed constant value of μ through a given temperature range. This discrepancy between the results and those of authors cited probably is due to the fact that they have calculated the value of μ on a graph with too small scale ordinates to account in full for the experimental accuracy of the velocity coefficients. It is suggested that a careful examination be made of all instances in which critical temperatures have been claimed in the literature.

4340

Further Observations on Latent Tolerance in Diabetics.

R. B. GIBSON.

From the Pathological Chemistry Laboratory, University Hospital, Iowa City.

A recent report on the stimulating effects on carbohydrate tolerance of successive short periods of a dietary high in sugar and low in protein and salt, with added insulin dosage, included data obtained on 2 mild cases in which improvement was maintained without interruption of the high sugar regime after withdrawal of insulin. Substitutions and additions were made to the diet of one of the patients, a young man 26 years old, until it was essentially normal in character . His condition was followed for 6 months without recurrence of the diabetic status. The present study is an attempt to determine how patients who were formerly insulin cases would react to an essentially similar procedure.

H., a student, age 20 years, was discharged from the hospital

---

[9] Euler, H. V., and Laurin, I., *Z. physiol. Chem.*, 1919, cviii, 64.

[10] Spohr, J., *Z. physik. Chem.*, 1888, ii, 194.

Nov. 26, 1928, on a maintenance diet with 12 units of insulin daily; his last blood sugar was 155 mgm. Thereafter he was managed as an out-patient, reported at short intervals to the laboratory, and received his meals from the therapeutic diet kitchen. He was able to discontinue insulin 3 weeks after his discharge after reductions to 7 units and 5 units daily. A high sugar diet period with insulin was started Jan. 4, 1929. Insulin was reduced daily until with-

TABLE I.

H., weight 63.8 kg.; medical service No. C 7918. Admitted Nov. 20, 1928; discharged Nov. 26, 1928.

Date	Blood sugar, mgm.		Insulin, units	Diet	Weight
	9:30 a. m.	2:30 p. m.			
					kg.
1/ 4	100		none	No. 65*	65.1
5	82	62	38 (18-10-10)	H.S.*	
6	50		22 (10- 5- 7)	H.S.	
7		140	13 (7- 0- 6)	H.S.	65.1
8	77		11 (6- 0- 5)	H.S.	
9		128	8 (5- 0- 3)	H.S.	
10	100		3 (3- 0- 0)	H.S.	65.0
11		108	discontinued	H.S.	64.5
12	96			H.S.	
13	110			H.C.*	
14		121		H.C.	
16		129		H.C.	
19	93			H.C.	
21	110			H.C.	
24	130	110		H.C.*	
29		172		H.C.	65.3
30		138		H.C.	64.7
31		120		H.C.	
2/ 1		208		H.C.	
2	102			H.C.	
3	93	151		H.C.	
4		96		H.C.†	
5		100		H.C.	
6	96			H.C.	
7	105	133		H.C.	
8	105			H.C.	
9	80			H.C.*	
10	86	110		H.C.	
11	130	127		H.C.	
12	102			H.C.	
13	100	155		H.C.	
14	110	151		H.C.	
15	96	105		H.C.	
17	94			H.C.	64.0
18	101	133		H.C.	
19	96			H.C.	

*P. 65 gm., C. 65 gm., F. 162 gm., Cal. 2045; H.S. is approximately P. 46 gm., C. 205 gm., F. 117 gm., Cal. 2100; initial substitution Jan. 13, and H.C. on Jan. 24 was P. 55 gm., C. 209 gm., F. 121 gm., and on Feb. 9 P. 68 gm., C. 199 gm., F. 114 gm.

† Breakfast increased and noon meal decreased, each by a third, from this day on.

drawn on Jan. 11. As this summary is submitted, he has been 40 days on the high sugar and carbohydrate diet without insulin. Blood sugars 2 hours after breakfast have been normal, but afternoon determinations have been irregular and somewhat higher than the morning figures,

Two more patients have been studied. R., age 18 years, had been able to dispense with insulin as the result of successive short periods of high sugar diet. F., age 23 years, had been managed carefully over a period of 2 years. Both responded satisfactorily for a time on high sugar diet when insulin was withdrawn, 11 days for R. and 16 days for F., until increasing and irregular blood sugars indicated that they be returned to the previous diabetic diet. When the diabetic condition was stabilized, the high sugar diet was given again; it was more successful so far as low blood sugar values were concerned, but could be continued no longer than before. A third attempt with both subjects was much less effective, though an increased hypoglycemic response to small insulin dosage was evident; this was probably an influence of the previous high sugar periods.

These results are in accord with the idea earlier suggested that a latent tolerance obtains in many cases of diabetes mellitus. Efforts to restore a normal tolerance, especially in young adults, seem well justified.

4341

Integumentary Pigmentation in Frog During Metamorphosis, with Especial Reference to Tail Skin Histolysis.

V. F. LINDEMAN. (Introduced by O. M. Helff.)

From the Zoological Laboratory, State University of Iowa.

Several types of larval skin transplantation experiments were made in an attempt to determine the local specificity of frog integument during metamorphosis when transplanted to foreign regions. The grafts involved skin from the back, belly, side and tail. Observations were made with respect to specificity of the integument for pigmentation and histolysis.

Autoplastic and homoplastic larval skin grafts showed local specificity for pigmentation upon larval transformation. All grafts invariably developed the pigment pattern during metamorphosis characteristic of the region from which they had been removed. Skin transplanted from back to side, or side to back, developed normal spotting characteristic of the region from which they had been taken. Skin transplanted from back to belly and vice versa, produced its normal spotting in the new position.

It is concluded that the integument of the frog *(Rana pipiens)* is specific for pigmentation and the factors responsible for the development of the normal pattern, during metamorphosis, are contained within the skin itself. Moreover, it may be concluded that certain factors within the blood stream are active or become active at a certain stage of metamorphosis and might well be conceived as being responsible for the rapid development of the latent pigmentation pattern present in the various areas of the integument.

Similar transplants involving an interchange of tail skin with skin from the back, suggested a specificity of tail skin toward histolysis. Conversely, skin transplanted from the back to the tail undergoes normal development during metamorphosis and develops the pigmentary pattern characteristic of the region from which it was taken. Instead of such grafts histolyzing and being absorbed due to their contact with atrophying tissues of the tail during metamorphosis, as might be expected, they invariably remained healthy and developed the normal pigment pattern characteristic of back skin. Moreover, the pigmentary changes of such grafts were found to take place simultaneously with the spotting of the back integument. Histological examination showed no signs of cellular histolysis, the integument having the appearance of normal back skin in every respect.

The reciprocal graft, however, in which tail skin was transplanted to the back underwent pronounced degeneration during metamorphosis and presented all of the typical macroscopic signs of histolysis. As metamorphosis progressed, the grafts were resorbed simultaneously with the resorption of the integument of the tail. Histological examination revealed cellular histolysis identical to that typical of histolyzing tail integument during the atrophy of the tail.

The results of the present work serve to throw light on the possible mechanism of tail skin histolysis as it occurs during normal atrophy of the tail during metamorphosis. It has been suggested by Barfurth[1] and Bataillon[2] that atrophy of the tail is induced primarily by the rapid growth of the urostyle during metamorphosis, which serves at least partially to occlude the blood supply to the tail. This, according to Bataillon[2] and Morse[3] would be a possible explanation for the condition of acidosis which occurs in the tissues of the atrophying tail. Morse, furthermore, points out that the acidosis present is sufficient to bring about autolytic reactions resulting in the atrophy of the tail. More recently, however, Helff[4] has shown that the urostyle cannot be the fundamental causative factor involved since typical tail atrophy was found to occur in animals in which the anlagen of the urostyle had been extirpated prior to metamorphosis.

---

[1] Barfurth, D., *Arch. f. miki. Anat.*, 1887, xxix, 1.

[2] Bataillon, E., *Ann. de Universite de Lyons*, 1891, II.

[3] Morse, W., *Biol. Bull.*, 1918, xxxiv, 149.

The results of the present work tend to support Helff's contention. The fact that tail integument transplanted to the back undergoes normal histolysis without the presence of adjoining atrophying tissue certainly requires some other explanation. Moreover, the fact that integument from the back transplanted to the tail never undergoes histolysis during tail atrophy is a negation of the idea that tail skin undergoes histolysis during metamorphosis due entirely to its association with the atrophying musculature beneath. It is concluded that 2 factors probably determine the histolysis of tail integument, one of which lies within the skin itself and is specific, and the second, within the blood stream, which becomes functional in inducing histolysis of tail integument at a certain stage of metamorphosis.

4342

Influence of Superheating on Antirachitic Properties of Irradiated Foods.

AMY L. DANIELS AND DOROTHY JORDAN.

From the Iowa Child Welfare Research Station, State University of Iowa.

There is some evidence that superheating destroys the antirachitic effect of irradiation.[1] In order to test this point further we have compared over a period of 10 weeks the growth performance of rats which were receiving a rachitic ration[2] with that of animals receiving (1) similar rations which had been irradiated; and (2) the irradiated ration which had been subsequently superheated. This rachitic ration which consisted of a mixture of grains was finely ground, mixed with distilled water and heated until it was of a thick gelatinous consistency. Since even at 15 pounds pressure mixtures of this consistency seldom reach throughout temperatures above 97° C., it was thought that results with these rachitic rations might not be valid. Therefore, we have tested the effects of the addition to the ration of (1) boiled milk; (2) irradiated boiled milk; and (3) irradiated milk which was subsequently autoclaved.

---

[4] Helff, O. M., *Anat. Rec.*, 1928, Dec., 39.

[1] Honeywell, H. E., Dutcher, A. R., and Dahle, C. D., *J. Biol. Chem.*, 1927, lxxiv, 77.

[2] McCollum, E. V., Simmonds, Nina, Parsons, H. T., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1920, xlv, 333.

In order to test further the effect of superheating on the antirachitic properties of milk, we have used in the rachitic ration comparable amounts of evaporated milk, as well as evaporated milk supplemented with calcium phosphate, thereby ruling out the influence of the possible calcium deficiency of the evaporated milk.

The rickets ration was irradiated in thin layers (1/8 inch) for ½ hour at a distance of 2 feet. Part of this was then autoclaved at 15 pounds for 6 hours. The milk was irradiated in ¼ inch layers for 20 minutes at a distance of 2 feet. Before autoclaving this was diluted with distilled water and heated at 15 pounds pressure for one hour.

The results of the investigation indicate that superheating, at least to the extent involved in the experiment, does not affect the antirachitic properties of food. This is shown not only by the animals which received the irradiated milks, but by those receiving the evaporated milk as well. The average gains per rat per week in the 2 groups fed the irradiated milks were comparable, and somewhat greater than those of the groups receiving the non-irradiated milks.

TABLE I.

Comparison of Growth of Animals Receiving Irradiated, and Irradiated Superheated Foods.

Ration	No. of animals	Initial weight Av.	Wt. after 10 wks. on diet Av.	Av. gain per rat per week	Remarks.
		gm.	gm.	gm.	
Control*	6	47.6	72.2	2.46	
Irradiated	2	51	151	9.8	4 females pregnant—not included.
Irradiated autoclaved	6	47	147	10.0	2 females bore young at 12 and 13 weeks respectively.
20 cc. milk boiled	6	45	101.2	5.62	One died 10th week.
20 cc. milk boiled†	6	58	115.2	5.7	One died 7th week.
20 cc. irradiated milk	6	42.3	113.2	7.13	One died 7th week.
20 cc. milk irradiated autoclaved	6	43	117.3	7.43	
10 cc. evaporated	6	52.3	134	8.2	
10 cc. evaporated + 0.2 gm. $Ca_3(PO_4)_2$	6	46.6	150	10.36	

*The control rachitic ration used in each experiment was Ration 2249, McCollum, E. V., Simmonds, N., Parsons, H. T., *et al.*, *J. Biol. Chem.*, 1920, xl, 333. The amount of milk specified was added to 100 gm. of the ration.

†Duplicate test started when animals were older, and showing similar results.

The animals receiving the evaporated milk made even better gains than those receiving the irradiated boiled milk, due in all probability to the difference in the antirachitic potency of the original milk. The better weight gains of the animals receiving the calcium phosphate additions are in accord with theory, since in the rations insufficient milk was used to supply the optimum amount of calcium.

Illinois Branch.

Northwestern University, February 28, 1929.

4343

Culturing Bovine Tubercle Bacilli.

MAX EVANOFF AND H. C. SWEANY.

From the Research Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.

The studies on culturing tubercle bacilli reported by Sweany and Evanoff[1, 2] have been applied to material from tuberculous bovine lymph nodes. Seventeen specimens were treated in various ways and inoculated on 3 types of media: the cream-egg; the milk-egg, and glycerine potato medium of Corper. The material was macerated in salt solution and inoculated directly; treated with 3% sodium hydroxide; 3% hydrochloric acid; and 6% and 12% sulphuric acid respectively.

All specimens grew directly on the cream medium, but none grew on media containing fresh glycerine. This was observed years ago by Park[3] and others. One specimen grew well by direct inoculation but scarcely at all after treatment. Strains vary greatly in their resistance to caustics. The more saprophytic strains appear to be less able to resist the effects of caustics.

The percentage growth following the use of various caustics is as follows: Of those treated with 3% HCl 89% of the tubes were positive; with 3% NaOH, 83.2% were positive; with 6% H_2SO_4, 78.7% were positive, and with 12% H_2SO_4 71.6% were positive. There was no serious contamination.

The direct smears grew 4 times out of 17.

Summary: 1. A direct culture method is described for obtaining growth and identifying tubercle bacilli from bovine material. 2. The bovine organisms isolated grow directly on cream-egg-milk medium, but not on glycerine-egg-milk medium. 3. Direct inoculation and treatment with 3% HCl and 3% NaOH are recommended for more complete results.

---

[1] Sweany and Evanoff, *Am. Rev. Tuber.*, 1928, xvii, 47.

[2] Sweany and Evanoff, *Am. Rev. Tuber.*, 1928, xviii, 661.

[3] Park and Krumweide, Dept. of Health, N. Y. City, 1916.

4344

A Method for Determining the Hardness of Nutrient Agar.

W. J. NUNGESTER. (Introduced by A. A. Day.)

From the Department of Bacteriology, Northwestern University Medical School.

The importance attached to details of colony forms in the work of recent years dealing with microbic dissociation, makes it imperative to recognize and control extrinsic factors affecting the structure of colonies. One such factor is the hardness of nutrient agar. Media prepared with agar from various sources has been found to cause detectable differences in the structure of the RS colony type of *B. anthracis.* This fact suggested the need of a method for determining the hardness of agar. Whipple[1] in 1902 studied the hardness or "spissitude" of gelatin. He also made a few tests on the spissitude of varying concentrations of agar. His method consisted in determining the depth a plunger 10 mm. in diameter would sink under a weight of 150 gm. in 5 seconds. The reciprocal of this value he termed spissitude. The sinking of the plunger was read without amplification.

The author has measured the hardness of agar by noting the time required for a brass plunger 0.500 cm. in diameter to sink into the agar 0.1 mm. under a given weight. The apparatus for accomplishing this consists of a vertical plunger attached to a horizontal lever in such a manner that one arm of the lever serves as a pointer against a scale, each unit of which represents 0.1 mm. of vertical motion of the plunger. The other arm, to which the plunger is attached near the fulcrum, serves as a beam on which a weight can be variously placed, thus altering the weight applied on the agar by the plunger. Making use of the mechanical features of an old microscope the above unit was mounted in place of the barrel. This facilitates lowering the plunger to the agar surface. When contact of the plunger and agar has been carefully established, a release is tripped, the weight of the system is applied through the plunger to the agar and the time for the plunger to sink 0.1 mm. is measured. The hardness of the agar is then expressed in units of hardness. The unit tentatively suggested is equal to $T \times W/100$ where T is the time in seconds required for the plunger to sink 0.1 mm., W is the weight in grams exerted on agar by the plunger, 0.500 cm. in diameter, and 100 is a divisor to eliminate non-significant digits in the product $T \times W$.

---

[1] Whipple, George C., *Tech. Quar.*, 1902, xv, 127.

Certain precautions are essential in order to obtain comparable results. (1) The agar must be poured at a stated temperature. (2) A uniform thickness of agar is necessary. This is insured by pouring 20 cc. of melted agar into petri dishes of uniform diameter (9 cm.) with a minimum of crowning in the bottoms. (3) Readings should be made at a uniform temperature 25±2° C. (4) The weight applied should be adjusted in order that the plunger will sink 0.1 mm. in less than 100 seconds and in not less than 40 to 50 seconds. The latter limit is to be particularly emphasized for more rapid sinking of the plunger is often accompanied by a cracking of the agar with resulting inaccurate readings.

Seven samples of various commercial agars were dried at 100° C. for 1.5 hours under a 28 inch vacuum. Two per cent solutions in distilled water were then prepared, dissolved by autoclaving, poured at 90° C. and tested after standing 24 hours. The results (Table I) indicate a wide variation in the hardness of agar prepared from various sources of dried agar.

TABLE I.
Hardness of Various Commercial Agars (2%).

Sample	1	2	3	4	5	6	7
Weight	4.6	4.6	51.9	21.0	12.2	9.2	29.6
Time	55.1	100.1	66.0	92.7	63.5	63.8	58.7
Hardness	2.5	4.6	34.2	19.5	7.7	5.9	17.4

In a similar manner the hardness of various concentrations of a sample of agar were tested. The results are given in Table II.

TABLE II.
Hardness of Various Concentrations of Agar.

Concentration	3.0%	2.5%	2.0%	1.5%	1.0%
Weight	120.0	91.2	51.9	34.4	12.2
Time	94.5	69.6	76.0	56.8	70.0
Hardness	113.4	63.5	39.4	19.5	8.5

Differences of 10 units in the hardness of nutrient agar has been found to affect the colony form of the RS type of *B. anthracis*.

4345

Experimental Hyperthyroidism and Sympathetic Irritability.

BEULAH CHAMBERLAIN. (Introduced by A. B. Luckhardt.)

From the Physiological Laboratories of the University of Chicago.

The object of this experiment was to make a study of the results of Goetsch's "Epinephrin Hypersensitiveness Test" upon dogs rendered experimentally hyperthyroid by feeding desiccated thyroid.

Goetsch's[1] test is based upon the theory that an oversecretion of the thyroid hormone renders the sympathetic nervous system more sensitive to the action of epinephrin. His method is as follows: In a patient at rest such subjective and objective manifestations as nervous symptoms, temperature, pulse, size of pupil, blood pressure, respiration, etc., are noted. Five-tenths cc. of 1-1000 epinephrin is then injected subcutaneously and deeply and the same observations are repeated. Goetsch states that there is usually an early rise in systolic and fall in diastolic pressure, a rise in pulse rate, and an increase of subjective symptoms, if the patient is hyperthyroid. There is no reaction whatever in other patients. Tests were made upon patients with known exophthalmic goitre to test the method, and the results were positive, parallel to the severity of the cases. Saline injections gave no reaction.

Luckhardt and Koppányi,[2] working with dogs, found that subcutaneous injections of epinephrin caused a rise in blood pressure in normal animals if massage of the area followed injection. The reaction was decreased by deep anesthesia and increased by peripheral dilatation (as from local injection of $Na\ No_2$). Light paraldehyde anesthesia or deep morphine analgesia did not interfere. These facts were made use of in our experiment.

The present experiments were performed in two series. In Series 1, 28 small dogs were used: 14 normal and 14 dogs previously made hyperthyroid by feeding desiccated thyroid—1 gm. per kilo body weight per day for one week. The dogs were lightly anesthetized with paraldehyde and the blood pressure recorded from the carotid upon a smoked drum. Two to 3 cc. of epinephrin were injected subcutaneously over the thorax and the area lightly massaged every minute until the first response, then at 10 minute intervals for 2 hours. Controls were made with saline. Averages of the results were calculated for the 14 normal and the 14 hyperthyroid dogs, as follows:

---

[1] Goetsch, Emil, *Penn. Med. J.*, 1920, xxiii; *N. Y. State J. Med.*, 1920, 1921, 1922.

[2] Luckhardt and Koppányi, *Am. J. Physiol.*, 1927, lxxxi.

TABLE I.

	Normal	Hyperthyroid
Average time before first response	9.5 min.	9.4 min.
,, height of first response	17.4 mm. Hg.	16.6 mm. Hg.
,, time before highest response	51.6 min.	38.3 min.
,, height of highest response	36.6 mm. Hg.	35.8 mm. Hg.
,, ,, of all responses	22 ,, ,,	25 ,, ,,

Control injections of saline were negative in all cases.

Although these results seemed consistently negative, we were not entirely satisfied, because of possible individual differences in dogs and in depth of anesthesia. We therefore performed a second series of experiments, in which 6 dogs were used. The animal was operated upon aseptically under ether to isolate one carotid and insert a three-way cannula. The dog was then allowed to recover for at least 1 hour from the ether, and was made analgesic with one-fourth grain of morphine sulphate. He then lay quietly upon the table, and a blood pressure record was made from the isolated carotid. At intervals intravenous injections of 1-500,000 epinephrin were made in doses of 1/10, 2/10, 5/10 and 1 cc.; blood pressure response was recorded upon the drum. The dog was then allowed to recover and fed thyroid, 1 gm. per kilo per day for 1 week, after which the same procedure was repeated, using the other carotid. Controls were made upon 4 dogs to compare the epinephrin reaction when one and when both carotids were ligated. An average of the results from the dogs in the normal and in the hyperthyroid state was as follows:

BLOOD PRESSURE RISE FOLLOWING EPINEPHRIN.

	0.1 cc.	0.2 cc.	0.5 cc.	1.0 cc.
Average of 6 dogs, normal	7 mm. Hg.	11.6	18.3	27.3
Average of same dogs, hyperthyroid	7 ,, ,,	9.3	15.3	25.0

The controls comparing the reactions when one or both carotids were ligated showed no difference.

Kunde's work[3] with thyroid medication has demonstrated the fact that dogs fed with 1 gm. per kg. body weight of desiccated thyroid per day for one week are unquestionably hyperthyroid. According to Goetsch's theory, such dogs should show greater sensitiveness to epinephrin than normal dogs would, or than the same dogs would before having been made hyperthyroid. Our results, however, do not support the theory, since they show no increase in blood pressure response to epinephrin in the hyperthyroid groups over that in the normal groups.

---

[3] Kunde, M. M., *Am. J. Physiol.*, 1928, lxxxii, 195.

4346

Reciprocal Inhibition During One Type of Pupillary Dilatation.

ZACHARY A. BLIER. (Introduced by A. B. Luckhardt.)

From the Physiological Laboratories of the University of Chicago.

In this investigation cats and rabbits under light ether anesthesia were used. The cervical sympathetic nerves were isolated on both sides of the neck, and the sciatic nerves were so prepared that their central ends could be stimulated with a tetanizing current. An attempt was made to keep the degree of anesthesia, as well as the intensity of the illumination, constant.

One sciatic nerve was stimulated and a dilatation of both pupils was obtained. The cervical sympathetic on one side of the neck was now sectioned and the central end of the sciatic stimulated with the same strength of current. A *bilateral* pupillary dilatation was again obtained, but the extent of dilatation seen in the eye, which had its cervical sympathetic cut, was less than that seen in the normal eye. In some animals a 1% solution of pilocarpine was instilled into the partially denervated eye. After the drug produced its maximum myosis the sciatic was stimulated as well as the peripheral (head end) of the cervical sympathetic. In the pilocarpinized, partially denervated, eye no effect was obtained from stimulation of the sciatic nerve, while stimulation of the peripheral end of the cervical sympathetic produced a maximum mydriasis.

In other animals the pilocarpine instillation was omitted, and 1% atropine substituted in its stead. In those animals which had received pilocarpine the atropine was later administered. In both sets the results were identical. When the atropine produces its maximum effects by paralyzing the endings of the oculomotor fibres, we can say the pupil is in a position of rest, that is, both sets of nerves are "sectioned", one by drugs and the other anatomically, and the muscles assume their position of rest. Stimulation of the sciatic nerve now produced the usual dilatation of the normal eye, but the pupil of the completely denervated eye remained entirely quiet and unaffected. The size of the normal dilated pupil exceeded that of the opposite one. The remaining cervical sympathetic was now cut and the pupil became smaller than the atropinized one. Stimulation of the sciatic nerve now produced a dilatation on that side, but the size of the dilated pupil did not equal that of the atropinized one.

The dilatation seen in response to sciatic stimulation after section of the cervical sympathetic is interpreted as being due to a diminution in the tone of the pupillo-constrictor muscle. This is similar to the reciprocal inhibition studied by Sherrington for striated skeletal muscle. There are, however, two striking differences. Sherrington's original study was performed, as alluded to above, on striated skeletal muscle, but we are here dealing with smooth muscle. Secondly, it is to be noted that the reciprocal relaxation is here not complete, while Sherrington found that the relaxation of the antagonistic muscle groups equalled the post mortem lengthening. This was especially true when he used strong stimulating currents. In no case could we produce a complete relaxation of the constrictor fibers, regardless of how strong the stimulating current was. This statement is supported by the fact that the dilatation observed in the sympathectomized eye did not equal that of the denervated atropinized eye, where presumably the constrictor fibres are completely at rest. The possibility of crossed fibres of the cervical sympathetic is ruled out, because when the dilatation after section of the sympathetic was obtained in the second eye, the other sympathetic was already sectioned.

It may also be added that when the normal eye dilates in response to the pain stimulus, the factor concerned with the dilatation is the radial musculature primarily; although the constrictor fibres act as physiological synergists by relaxing, they do not do so completely, and it is necessary for the radial fibres to stretch them to produce a mydriasis.

A typical protocol is appended:

Procedure	Right Eye	Left Eye
Normal	3mm.	3mm.
Stimulate right sciatic	7	7
Cut right cervical sympathetic	3	5
Stimulate sciatic	5	8
Instill 1% pilocarpine in right eye	1	4
Stimulate sciatic	1	8
Stimulate right cervical sympathetic	9	4
Put atropine in right eye	6	4
Stimulate sciatic	6	8
Cut left cervical sympathetic	6	3
Stimulate sciatic	6	4
Instill atropine in left eye	6	6

The author wishes to take this opportunity to thank Dr. Arno B. Luckhardt for suggesting this piece of work, and for his continuous help.

4347

The Effects of Pancreatectomy in Ducks.

I. J. SEITZ AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The observations of various investigators in the field of experimental avian *diabetes mellitus,* while not agreeing in all respects are, nevertheless, unanimous in that the removal of the pancreas from chickens and ducks results in a condition which is essentially different from a von Mehring Minkowski[1] diabetes in the dog. Weintraud[2] reported glycosuria in only a small proportion of his depancreatised ducks. Kausch[3] stated that the blood sugar changes in a large series of depancreatised ducks varied from no increase at all in a few birds to an extreme hyperglycemia in the majority, but that there was no constant relation between the incidence of hyperglycemia and glycosuria in his series, and that the livers of these ducks were practically glycogen free even after heavy feeding. In a similar study on chickens, Giaja[4] reported that the removal of the pancreas resulted in no glycosuria and only a slight increase in the sugar content of the blood.

More recently, Koppányi, Ivy, Tatum and Jung[5] and Redenbaugh, Ivy and Koppányi[6] in studies on depancreatised chickens observed that while the resulting hyperglycemia was temporary it was always accompanied by glycosuria. Extracts from the livers of these chickens gave a positive iodine test for glycogen. They observed also that the pancreas of normal chickens yields approximately as much insulin as the pancreas of calves is reported to, and that the liver and kidneys of normal chickens contain relatively large amounts of insulin. In view of the atypical diabetes which results in depancreatised fowls, these workers[5] offered two possible explanations: either, that after the removal of the pancreas from fowls some other organ, probably the liver, may develop the ability to regulate carbohydrate metabolism in some unknown manner, or, that insulin production is not solely specific for the pancreas though the pancreas would appear to be the main producer of this hormone.

---

[1] v. Mehring, J., und Minkowski, O., *Centralbl. f. Klin. Med.*, 1889, x, 393.

[2] Weintraud, W., *Arch. f. Exper. Path. u. Pharm.*, 1894, xxxiv, 303.

[3] Kausch, W., *Arch. f. Exp. Path. u. Pharm.*, 1896, xxxvii, 274.

[4] Giaja, J., *Compt. Rend. de Soc. Biol.*, 1912, lxxiii, 102.

[5] Koppányi, T., Ivy, A. C., Tatum, A. L., and Jung, F. T., *Am. J. Physiol.*, 1926, lxxviii, 666.

[6] Redenbaugh, H. E., Ivy, A. C., and Koppányi, T., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 756.

In the present study on 6 normal and 10 depancreatised ducks, we found that immediately after the pancreatectomy, the blood sugar level was definitely elevated but that it gradually returned to within normal limits of variation within a week or 10 days, and that 2 months after the pancreatectomy, the liver and muscle tissue gave a positive iodine test for glycogen. We found that extraction of liver, kidney and muscle tissue of normal ducks by the Fisher[7] modification of the Doisy, Somogyi, Shaffer[8] method of insulin preparation yielded a substance which lowered the blood sugar of fasted normal rabbits, but that similar extracts from depancreatised ducks did not.

Quantitative studies are now being made to ascertain the nature of carbohydrate metabolism in depancreatised ducks.

4348

Experimental Intestinal Obstruction.

HILGER P. JENKINS. (Introduced by L. R. Dragstedt.)

From the Department of Surgery, University of Chicago.

To determine the rôle of biliary, pancreatic, and duodenal secretions in the rapid death of acute intestinal obstruction an experiment was devised which permitted these three secretions to be short circuited into the bowel below the point of obstruction. This was done by operations on dogs in 2 and 3 stages, the latter giving the better results. Before producing the obstruction which was done as the last stage, the jejunum just beyond its junction with the duodenum was sectioned and the proximal portion anastomosed to the ileum. Then a pylorectomy was done and the distal portion of the jejunum was anastomosed to the stomach. In this way the duodenum emptied into the ileum, and the stomach into the jejunum.

Following this procedure the animals often lost weight, had a diarrhea, became progressively weak, and died of exhaustion or bronchopneumonia. Two developed chronic ulcers of the jejunum. Those which survived and were in good condition were obstructed by sectioning the bowel above the anastomosis in the ileum and invaginating the stumps with a purse string suture. Six dogs with obstruction produced in this manner lived from 12 to 33 days. With the obstruction 1 foot from the stomach the dog lived 12 days. At 20 inches the duration was 29 days. At 2½ feet the duration was 17 and 33 days. Three feet from stomach was lethal in 27 days. At 5 feet it was 17 days.

---

[7] Fisher, N. F., *Am. J. Physiol.*, 1923, lvii, 57.

[8] Doisy, E. A., Somogyi, M., and Shaffer, P. A., *J. Biol. Chem.*, 1923, lv, 31.

The behavior of these animals was not at all like ordinary obstructed animals which die in 2 to 7 days. They were quite lively, would walk and sometimes run. The average intake was 200 to 400 cc. of water a day to which glucose and sodium chloride were often added. Vomiting occurred about every second to third day. The urine output averaged 100 to 250 cc. per day. Small black or brown stools were passed on the average of every fourth or fifth day. The weight of these dogs gradually decreased to half the original weight, the average loss being 0.24 to 0.37 kg. per day. Studies of the blood chemistry showed a marked fall in the chlorides, an increase in CO_2 capacity, and rise in non-protein and urea nitrogen which became very marked just before death. It is interesting to note that the N.P.N., Urea N., and CO_2 decreased during the first week of obstruction in some cases. When autopsied death was found to be due to some complication in half the dogs. In one bronchopneumonia was found, in another intussusception with perforation and general peritonitis, in another intussusception and obstruction of the ileum which produced a condition identical to a closed duodenal loop. In all cases the obstructed jejunum was very noticeably distended especially at its distal end.

Conclusions: These experiments seem to show that the biliary, pancreatic, and duodenal secretions have a very important rôle in the rapid death of acute intestinal obstruction. One explanation is that in these dogs the pancreatic, duodenal, and biliary secretions do not flow into the obstructed loop and there produce a toxin which is absorbed in ordinary cases of obstruction. Or another explanation is that these secretions are reabsorbed in the ileum, a process which is necessary for life.

4349

The Glycogen Content of Muscles in Tetanus Contracture.

H. A. DAVENPORT AND S. W. RANSON.

From the Institute of Neurology, Northwestern University Medical School, Chicago.

Local tetanus was produced in one hind limb of experimental animals (rats, guinea pigs and rabbits), and the glycogen content of flaccid and contracted gastrocnemii compared. Time intervals of 2 to 58 days were allowed to elapse from time of injection of tetanus toxin to sacrificing the animal.

In general, less glycogen was found in the shortened muscle than in the flaccid gastrocnemius from the opposite (uninjected) side. Two rabbits were allowed to survive for over six weeks and when sacrificed the tetanus gastrocnemii although still in contracture contained more glycogen than their functioning companions. Guinea pigs which survived for seven weeks still showed reduced glycogen in tetanus muscles unless they had regained function. In the latter case (one animal) the glycogen content was the same on both sides. Rats showed very little change in glycogen from the normal.

In view of the variability of results it was concluded that the changes in glycogen were indirectly associated with the phenomenon of contracture. This view was strengthened when it was found that contractures produced in the hind limbs of cats by section of the dorsal roots of spinal nerves were not associated with changes in glycogen content of the shortened muscles.

Our observations on the glycogen changes in tetanus muscles are in agreement with those made by Wertheimer[1] on guinea pigs.

4350

Reaction of Gall-Bladder to Faradic Stimulation of Stomach.

C. L. BIRCH AND E. A. BOYDEN.

From the Departments of Medicine and Anatomy, University of Illinois, College of Medicine, Chicago.

In a previous publication attention was called to a case of spontaneous emptying of the human gall bladder during fasting, in which the patient reported the occurrence of hunger pangs during the interval in which cholecystograms were recording a marked reduction in size of the gall bladder.[1] This was interpreted as being in harmony with Boldireff's observations that during fasting, bladder bile is discharged into the duodenum synchronously with gastric hunger contractions.

---

[1] Wertheimer, E., *Arch. ges. Physiol.*, 1928, ccxxi, 139.

Very recently, we have observed rhythmic tonus changes of the gall bladder, in fasting cats, that are concurrent with rhythmic peristaltic contractions of the stomach. These observations, made on unanesthetized animals 7 to 10 hours after operation, were recorded by series of x-rays taken at intervals of 30 seconds—the gall bladder being visualized by roentgen-opaque oil, and the stomach by its content of air. The intervals between phases, as well as the duration of the contraction waves (1 to 3 minutes), seem to correspond to the periods of gastric hunger contractions described in the literature.

That these tonus waves of the gall bladder are induced by successive contractions of the stomach would seem to be verified by the following experiments. If insulated electrodes be sewed to the greater curvature of the *pars pylorica*, and the cat be allowed to recover from the operation, weak faradic stimulation of the stomach (½ milliampere) causes ring contraction of that organ and simultaneous contraction of the gall bladder. If a stronger current be used (6½ milliamperes) the lipioidol in the gall bladder may be shot up into the cystic duct, the contractile cycle of the gall bladder lasting from 1 to 3 minutes, depending on the strength of the contraction. This seems to indicate that the mammalian gall bladder is directly under control of reflexes originating in the gastro-intestinal tract.

But if the cat be given a meal of egg-yolk, and it be ascertained by x-rays that contraction and emptying of the gall bladder is taking place, then faradic stimulation of the stomach inhibits emptying of the gall bladder. This observation suggests the possibility that dysfunction and stasis of the human gall bladder may be induced by chronic inhibitory reflexes originating in a diseased gastro-intestinal tract, and that certain types of biliary pathology may be merely part of a syndrome involving interrelated portions of the digestive system.

---

[1] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 157.

Missouri Branch.

Washington University School of Medicine, February 26, 1929.

4351

Effect of Feeding Small Doses of Potassium Iodide on the Thyroid Gland.

S. H. GRAY AND J. RABINOVITCH. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In previous experiments reported from this laboratory it has been shown that the oral administration of 0.01, 0.05 and 0.1 gm. KI daily for a period of 15 to 20 days to normal guinea pigs results in a very marked proliferative activity of the thyroid epithelium,[1, 2] which, as estimated by the number of mitoses, was directly proportional to the size of the dose.[2] The smallest dose of iodide used in the earlier experiments in the guinea pig, corresponded approximately to a therapeutic dose used in the human being, but 0.05 gm. and 0.1 gm. exceed considerably the quantities used in patients. Yet, even when the largest doses of potassium iodide were used none of our animals showed any injurious effects; they usually gained in weight, and appeared perfectly normal in every respect. It was, nevertheless, of interest to study the effects of very small doses of potassium iodide upon the thyroid glands and the following experiments were therefore performed.

Twenty-two guinea pigs, weighing between 325 and 450 gm. were divided into 4 groups, the first of which served as controls and received no iodide; the other 3 groups were fed respectively with daily doses of 0.01, 0.001 and 0.0001 gm. KI for a period of 20 days, at the end of which time, the animals were killed and the thyroids removed and studied in a manner already described in the previous communications.

I. *Estimation*

---

[1] Gray, S. H., and Loeb, Leo, *Am. J. Path.*, 1928, iv, 257.

[2] Rabinovitch, J., *Am. J. Path.*, 1928, iv, 601.

Table I shows the results obtained, as far as the mitotic activity is concerned.

TABLE I. *Tabulation of Mitoses.*

	Control	0.0001 gm. KI	0.001 gm. KI	0.01 gm. KI
	72 232	486 288 56 48 520 S 2000*S	140 524 430 196 1018* 20*S 220 S	924 1814 1172 1296 160*S 906 S
Total average	152	566	507	1045
Average if figures with * are omitted	152	280	302	1224

(a) Control animals. Only 2 animals were used in the control group because the results obtained conformed closely to those observed in a very large number of control guinea pigs previously studied. The number of mitoses in the whole thyroid gland of these 2 animals were 72 and 232. These figures are within the control limits.

(b) Animals fed with 0.0001 gm. KI. The number of mitoses in this series of animals varied considerably. The figure 2000 found in our guinea pig is exceptionally high. When this number is omitted, the average number of mitoses is 280, which is slightly above the average of the control. However, at best this increase in the average of mitoses is very slight.

(c) Animals fed with 0.001 gm. KI. The mitotic activity found in this series also showed considerable variations. The average number of mitoses was 302, which is somewhat greater than in the controls although not very markedly so. This figure is obtained if we omit the 2 extremes (1018 and 20); otherwise the average exceeds that of the controls more decidedly.

(d) Animals fed with 0.01 gm. KI. The number of mitoses per gland in this group, with the exception of one (160), is much more constant than in the preceding 2 groups. We may omit this figure as an individual variation due to an unknown variable factor. The remaining animals present a marked increase in mitotic activity considerably greater than that found in the controls, or in the animals fed with smaller doses.

Although the feeding of 0.0001 and 0.001 gm. KI does not produce a pronounced increase in mitoses, it calls forth a slight increase above that found in the untreated control animal. This is especially true of some individual animals in which the increase was quite pronounced. When we omit from our average the extreme figures obtained (marked in the table by *) the number of mitoses in the animals treated with the very small doses of KI, approaches closely the upper limits obtained in the controls. A markedly stimulating effect of the iodide on the mitotic proliferation becomes quite noticeable when a dose of 0.01 gm. KI is used. These figures also indicate that the larger the dose of KI administered to the animal, the greater is the resulting mitotic activity.

Some of these experiments were carried out in the summer months of 1928, while others were carried out during winter months. The ones done during the summer months are designated in the table by the letter "S". The results obtained during the warm season are more irregular than those obtained at other times; in general, they have the tendency to be low in animals fed with KI. This latter conclusion is borne out also by additional experiments not included in this table.

II. Changes in the character of the acini, epithelium and colloid. The histological structure of the average control gland may be described briefly, as follows: The acini are of medium size, lined by low cuboidal epithelium, but variations occur in different parts of the gland. The colloid is solid, but occasionally shows areas of softening and may or may not be infiltrated with phagocytes. In the interstitial tissues are found at times lymphocytic or leucocytic accumulations, although these latter findings are unusual.

The structural picture of the thyroid obtained from animals fed with 0.0001 gm. KI is very similar to that found in the control. These findings therefore indicate that the thyroid gland of guinea pigs does not respond to doses as small as 0.0001 gm. KI.

When doses of 0.001 gm. KI were administered, the glands showed a greater response. The acini tended perhaps to become slightly larger and somewhat more irregular in shape, and the epithelium was on the average slightly higher, than in normal glands. The colloid showed more softening and was more densely infiltrated with phagocytes. Such a structure of the thyroid is very similar to that observed in cases in which larger doses of iodide (0.01-0.1 gm.) were used and where mitotic activity was very pronouncedly increased. These structural changes in the gland correspond to a certain extent, with the mitotic cell activity of the acinar epithelium, inasmuch as they are found only in such guinea pigs as have been fed doses of KI which on the average increase the mitotic activity, and inasmuch as a very low number of mitoses is not usually associated with a softening of colloid, increase in phagocytes and slight increase in the size of the acinus cells. But if 0.001 gm. KI is fed these changes may be visible even in cases where there is no definite increase in the number of mitoses, and there is moreover no direct parallelism between the doses of KI given, the increase in mitotic activity and the characteristic structural changes. The thyroid gland of normal guinea pigs may show marked structural variations which, in some cases, closely resemble the gland of animals fed with KI in that the colloid is soft and infiltrated with phagocytes while the epithelium is high and acini somewhat larger and of irregular shape.

Discussion. These experiments show that the oral administration of very small doses of KI (viz. 0.0001 gm. KI) to the normal guinea pigs for a period of 20 days, evokes no marked response on the part of the thyroid gland in so far as proliferation of the epithelium or structural appearance of the gland are concerned. When the dose is increased to 0.001 gm. KI, mitotic activity is still low, but there appear in the gland definite structural changes, found usually in gland of animals which have been fed larger doses of KI. These changes consist mainly in larger and more irregular acini, higher epithelium and softer colloid infiltrated with phagocytes. As small a dose as 0.001 gm. KI may stimulate the thyroid gland without necessarily causing an increased mitotic activity. This presents the first variation of what heretofore has been thought to be a parallelism: The greater the number of mitoses the more marked the other histological signs of thyroid activity.

When still larger doses of KI are administered, viz., 0.01 gm. KI, the number of mitoses in the gland increases considerably above the normal control. Associated with the rise in the number of mitoses, we also find the larger, irregular acini, the higher epithelium and softer colloid.

Summary. 1. The daily feeding of 0.001 gm. KI to guinea pigs does not increase the number of mitoses and does not affect the structure of the thyroid gland. 2. Feeding daily 0.001 gm. KI to guinea pigs produces only a slight increase in mitoses but at the same time produces an otherwise histologically active thyroid gland. 3. The feeding of 0.01 gm. KI stimulates the thyroid in all its histological phases.

---

* We are indebted to Dr. Leo Loeb for his helpful advice.

4352

Arterial Blood Pressure and Blood Flow in Skeletal Muscles as Influenced by Epinephrine.

CHARLES M. GRUBER.

From the Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

In this research unanesthetized cats were used. The spinal cord was cut in each case in the thoracic region. All operative work was done under general ether anesthesia after which the anesthetic was discontinued for 5 to 24 hours before the injection of epinephrine. In these animals dilute solutions of epinephrine in small amounts injected in the same way caused in some animals a fall, in others no change, and in still others a rise in blood pressure.

Small doses of epinephrine invariably caused an increase in the rate of blood flow in skeletal muscles irrespective of the change in blood pressure. Large doses of epinephrine rapidly injected intravenously caused an increase in blood pressure accompanied by a decrease in rate of blood flow in skeletal muscle. Following the rise the blood pressure fell below the normal level and simultaneously with it a marked increase in the rate of blood flow through the muscles was observed.

These results upon unanesthetized cats confirm the findings in anesthetized and decerebrated ones—in that epinephrine produces a dilatation of the blood vessels in one region of the body at the same time that it causes vaso-constriction in other regions, and the blood pressure recorded is the stronger effect minus the weaker.

4353

Effect of Infections on the Thyroid Gland.

J. RABINOVITCH AND S. H. GRAY. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Cole, Womack and Gray[1] and others[2, 3] described certain pathologic changes in thyroids of persons who suffered from acute or chronic infections, such as pneumonia or peritonitis. The glands were obtained at autopsy from individuals who succumbed to either an acute or chronic infection. The changes noted in the thyroid were of a two-fold nature, depending in each case on the acuteness or chronicity of the infection from which the patient died. Thus, where death was a result of pneumonia or acute peritonitis, the changes in the thyroid consisted mainly in desquamation of the epithelium lining the acini and softening of the colloid. When death of the individual was due to a more chronic type of infection, the thyroid did not show any epithelial desquamation, but rather a heaping up of the acinar cells with papillary projections into the alveoli, and increased scar formation. Very similar changes to those described above, were previously noted by Cole and Womack[2] in dogs in whom they had experimentally produced acute infections and toxemias.

---

[1] Cole, W. H., Womack, N. A., and Gray, S. H., to be published, *Am. J. Surg.*

[2] Cole, W. H., and Womack, N. A., *J. Am. Med. Assn.*, 1928, xc, 1274.

[3] See literature in references 1 and 2.

We thought it of interest to carry out similar experiments in guinea pigs, because the thyroid gland of guinea pigs has been very carefully studied in the laboratory under normal as well as under various experimental conditions and we are therefore well acquainted with the changes which take place in the thyroid of guinea pigs under various conditions.

We used 26 guinea pigs, in 16 of which an acute infection was produced by injecting fecal material into the peritoneal cavity. The time of development of an acute peritonitis and the consequent death of the injected animal was found to vary according to the quantity of injected material and site of injection. If the site of injection was high up in the abdomen, viz., in the region of the diaphragm, where absorption is more rapid, the animal died much sooner than when injected in the pelvis.

Chronic peritonitis was produced in 6 guinea pigs, by injecting repeatedly small doses of fecal material intraperitoneally for periods of time ranging between 2 weeks and 4 months. Added to the series of acute infections, was one animal that died of pneumonia and another that succumbed to an acute necrosis of the subcutaneous tissue of the abdomen.

Controls consisted of thyroids removed from normal animals killed in chloroform. In a very large series, the thyroids were removed and fixed immediately after death. Four guinea pigs were returned to their cages after death and allowed to remain there at room temperature for 4, 6 and 18 hours before the thyroids were removed and fixed.

I. Control series. Thyroids removed from animals immediately after death due to inhalation of chloroform and fixed in Zenker's solution, showed the following characteristics: The acini were of a variable size, ranging in dimension from small to intermediate and large. The lining epithelium was usually of a cuboidal type, although occasionally taller or lower than the average. The colloid was solid as a rule, but at times contained phagocytes that caused a partial or complete liquefaction and absorption of this substance. There was no evidence in these cases of any desquamation of the epithelium anywhere in the gland. In the interstitial tissue, there were occasionally lymphocytic or leucocytic infiltrations. The capillaries were sometimes congested and a moderate or even extensive increase in connective tissue was found in certain glands, particularly in the region of the larger vessels.

Glands removed from animals in which death occurred some time previously, showed some additional characteristics after death. The size of the acini and epithelium were the same as those described above. There was, however, present a definite but very slight desquamation of the epithelium, while the colloid was solid and showed a slight tendency towards softening. In animals in which the thyroids were removed 18 hours after death the desquamation of the epithelium was intense, and the colloid was somewhat softer in acini where desquamation was apparent.

II. The thyroid of infected guinea pigs. To this series belong 16 animals injected intraperitoneally with fecal matter, 10 of which were found dead in their cages; the thyroids in these instances were removed at an indefinite time following the death of the animal. The remaining six were found very acutely ill, and these were killed by chloroform and the thyroid removed and fixed immediately after their death. The thyroid of this latter series of animals displayed the following histological characteristics: 5 of these animals showed no desquamation of the acinar epithelium; the remaining one showed a definite desquamation. The size of the epithelium in every instance was cuboidal and the colloid hard, except in the animal where desquamation was noted, the colloid was soft.

In the large majority of cases the fecal matter was injected late in the afternoon. Ten of the injected animals were found dead early the next morning and were autopsied immediately. The time interval between death and autopsy could not have been more than a few hours. In 6 of the animals found dead, the thyroid acini showed marked desquamation of the epithelium; in 3 there was only a mild degree of desquamation, and in one desquamation was entirely absent. The epithelium in all these animals was cuboidal. The colloid, on the whole, was solid but there were occasional areas of softening, particularly in the acini that showed desquamated epithelium.

III. The thyroid of guinea pigs chronically infected. In this series we had 6 guinea pigs which had received 3 to 7 doses of fecal injections over periods ranging between 3 weeks and 4 months. Four of these were killed by chloroform and 2 were found dead. Histologically, none of the thyroids removed showed any desquamation. The acini were small to medium in size; the epithelium was cuboidal and the colloid was hard. Three of these glands had a cellular appearance, probably due to the crowding of small acini and to the apparent filling of the interacinar spaces with epithelial cells. The number of mitoses, however, showed no increase above the normal. In one of these animals, scars were found in the interstitial tissue.

Discussion: Desquamation of the epithelium and softening of the colloid which had been described in the human and dog thyroids after acute infections, occurred only in one of the 6 acutely infected guinea pigs that we killed. This picture was noted however in 9 of the 10 acutely infected animals that were found dead. These changes occurred earlier and to a more marked degree in the latter group of animals than in our control animals which were examined some time after death. Whether these changes are due to specific effects of the infection upon the thyroid gland during life, or whether they were caused by postmortem autolysis which was hastened and intensified because of the presence of an infection and toxemia, we are as yet unable to determine definitely. However, the fact that only one of the 6 acutely infected animals, that we killed when very ill, showed this desquamation, seems to support the second view. It must be remembered, however, that different species of animals as well as individual animals of the same species may react differently to the same experimental procedures.

In the cases of chronic infection, we found no desquamation of the epithelium and the colloid was hard; 3 of these animals also showed very cellular glands, and one animal showed scar formation. These facts are in agreement with the findings noted in the human and dog. However, we also found similar cellular glands and scar formation in 4 of the 16 acutely infected animals. Furthermore, in going over a large number of thyroid removed from normal guinea pigs and from guinea pigs fed with various substances and in which we could find no evidence of infection, we noted a fair proportion of cases presenting this same cellular appearance as well as scar formation. The former was especially found in a series of animals that were underfed[4] and in which there was no increase in cell mitoses.

Summary: 1. In 22 guinea pigs an acute and chronic peritonitis was induced by injecting fecal material intraperitoneally. 2. In only one guinea pig examined before death was any desquamation of epithelium and softening of the colloid noted. In guinea pigs found dead, post mortem desquamation occurred earlier in experimental infections than in controls. 3. In chronic infections the guinea pig thyroid presented no deviations from the normal.

---

[4] Rabinovitch, J., *Am. J. Path.*, 1929, v, 87.

New York Meeting.

New York Academy of Medicine, March 20, 1929.

4354

Return of Vision and Other Observations in Transplanted Amphibian Eyes.

D. N. BEERS. (Introduced by L. S. Stone.)

From the Department of Anatomy, Yale University School of Medicine.

Return of vision and various other observations were reported by Stone and Ussher[1] on replanted eyes in *Amblystoma punctatum* larvae. The present paper gives a report of similar observations based on 126 transplanted larval eyes in the same species. The methods of technique in operation were similar to those employed by the above mentioned authors. In nearly all cases right eyes were exchanged between pairs of larvae 22 mm. in length. Animals were killed at intervals of a few days from the time of operation until long after metamorphosis. Observations obtained of eyes in the animals while living have been correlated with those obtained from a microscopic examination in 62 cases.

Very little hemorrhage was encountered. Resorption of the eye, when it took place, could usually be correlated with known injuries to the bulb at the time of operation, or to prolonged use of anesthesia, or to too strong anesthesia. Slight reduction in the size of the transplanted eye was noted in most cases after operation, but usually the normal size appeared to be regained within 5 weeks. In fact the general macroscopic appearance of the transplanted eyes was so similar to that of the normal eyes in most cases that it was often difficult to differentiate between them. Shortly after operation the pupil of the transplanted eye showed some dilatation, but later approached normal size.

Return of circulation was first noted within the superficial vessels of the iris as early as 24 hours after operation in one case. The average time, however, was about the 4th day. Return of some ocular movement was observed as early as 7 days after operation, but in most cases this occurred much later. Pupillary and corneal reactions did not occur in any transplanted eyes until the animals had begun metamorphosis. This is always true of normal *Amblystoma punctatum* eyes.

---

[1] Stone, L. S., and Ussher, N. T., Proc. Soc. Exp. Biol. and Med., 1927, xxv, 213.

To test for return of vision in the transplanted eyes care was taken to eliminate olfactory, gustatory, tactile and mechanical disturbances such as jarring or touching the aquarium. The lure for eliciting visual response was a piece of red rubber impaled on a wire and moved about a tightly covered glass aquarium. The normal eye was excised in animals so tested. If the animal snapped at the lure and followed it about the bowl on several successive days, when tested by disinterested observers as well as the author, vision was considered present. Vision was not considered to have returned if the animal never snapped at the lure after trials made over a period of several weeks. Vision was found to have returned in one case, a larva, as early as 48 days after operation. Many tested after metamorphosis showed visual response through the transplanted eye. In some animals return of vision was not tested, for it was necessary to preserve the normal eye in a sufficient number of cases to supply controls for histological studies. For comparison, controls were used, *viz.*, animals (a) with 2 normal eyes present, (b) with 2 eyes extirpated, and (c) with 1 normal eye present and 1 extirpated.

Microscopic studies rarely revealed any changes in the lens. The ganglion-cell layer of the retina showed a single row of cells—a contrast to the double row of these cells in normal eyes. Occasionally there were small patches devoid of cells and in rare instances small areas contained a second row of pale-staining vesicular cells. This irregularity in the ganglion-cell layer was not noticed in the larval eyes before the first month after operation and it was not a constant finding until well after the second month. In a few cases of adult animals there were noted undulations and irregularities in contour involving the entire retina—a condition not observed in normal eyes. The stratum opticum and the rod and cone layers of the transplanted eyes in both adult and larval stages seemed to reveal no striking deviation from that of the normal eye. The optic nerve, in nearly all cases, showed an enlargement at its exit from the bulb where the severed ends of the nerve had come together. Proliferation of cells at the cut ends of the nerve could be seen as early as the 4th day after operation. After the 25th day, in nearly all of the transplanted larval eyes, the enlargement was present and the nerve itself extended to the chiasma and brain without a break in its continuity.

In a majority of cases studied that portion of the optic nerve extending from the enlargement to the brain seemed to be about normal in size, but tortuous and irregular. In no case after metamorphosis was the optic nerve lacking.

4355

Effect of Drying on Certain of the Virus Group.*

JAMES A. HAWKINS. (Introduced by James B. Murphy.)

From the Laboratories of the Rockefeller Institute for Medical Research.

It has long been known that certain of the virus group such as vaccine and rabies virus will withstand drying but as a rule there is considerable loss in virulence during the process. Murphy[1] showed that the chicken tumor agent was less damaged if the material was kept frozen during desiccation. Harris and Shackell[2] demonstrated that rabies virus frozen and dried lost little of its virulence and, furthermore, the deterioration was less rapid than when the tissues were dried by the usual methods.

The present study deals with the resistance of vaccine virus, virus III and *Herpes febrilis* virus to freezing and desiccation.†

The various tissues containing the viruses were mixed finely and spread in a thin layer in a sterile dish. This was placed in a vacuum jar over concentrated sulphuric acid and the pressure reduced to 5.0 mm. of mercury. The jar was immediately put in a freezing room at a temperature of –5.0° C. and left for 2 days. The tissues, thoroughly desiccated by this time, were removed, rubbed to a powder in a mortar and sifted through a fine meshed sieve to remove the larger particles. For the infectivity test the powder was taken up in Locke's solution and injected in the usual manner for the individual virus.

Vaccine virus, after the above treatment, when tested dermally and intradermally in normal rabbits, showed that the activity of the virus was practically unimpaired. The lesions produced arose as promptly and were almost as extensive as those produced by the control injections of fresh vaccine virus.

---

* This investigation was carried out by means of funds from the Rutherford Donation.

[1] Murphy, James B., *J. Exp. Med.*, 1911, xiii, 889.

[2] Harris, D. L., and Shackell, L. F., *J. Am. Pub. Health Assn.*, 1911, i, 52.

† We wish to acknowledge the assistance of Dr. Thomas Rivers, who supplied the viruses used and tested their infectivity after desiccation.

Virus III after drying in the frozen state proved almost as potent as the fresh virus in the production of the typical testicular lesions in rabbits.

Herpes febrilis vaccine subjected to the freezing and drying produced the typical *Herpes febrilis* reaction in rabbits, killing the animals in 5 days. The brains showed very many typical eosin-staining nuclear inclusions.

Viruses dried by this method and kept in sealed tubes in the icebox lose their virulence very slowly. There was no appreciable loss after one year under these conditions. This offers a convenient and economical method of preserving strains of viruses.

4356

Resistance of Causative Agent of Chicken Tumor to Certain Organic Solvents Compared with Vaccine Virus and Herpes Febrilis Virus.*

JAMES A. HAWKINS. (Introduced by James B. Murphy.)

From the Laboratories of the Rockefeller Institute for Medical Research.

Because of the theoretical importance of the chicken tumors, it seems desirable to continue the study of the properties of their causative agent from a number of angles with the hope that this may lead to an understanding of their nature. The present study deals with the resistance of the agent of Chicken Tumor I to the action of certain solvents and a comparison of this resistance with that of some of the filterable virus group.†

Experimental Procedure. The various tissues containing the active agents were treated as described in the previous paper to obtain dried materials. One half gram portions were transferred to long glass tubes containing 50 cc. of various organic solvents. The tubes were sealed by fusing the open end in a flame and were shaken for 6 days by a mechanical shaker. They were then opened and the emulsion of solvent and tissue was filtered through filter paper. The residue was washed with solvent twice, transferred to a petri dish and freed from solvent by evaporation. The clear filtrate was also placed in a petri dish and evaporated to dryness by passing a stream of cold, bacteria-free air over it. In each case a small amount of fatty material was left after evaporation. The residue after the extraction of the dried materials and the fatty substances extracted were taken up in Ringer's solution and tested for the potency of the agent.

---

* This investigation was carried out by means of funds from the Rutherford Donation.

† We wish to acknowledge the assistance of Dr. Thomas Rivers, who supplied the viruses used and tested their infectivity after the treatment with the various solvents.

Chicken Tumor I. The tumor producing activity of the residues after extraction with dry alcohol, toluol, or acetone was unimpaired. On the other hand, the material was inactivated by treatment with chloroform, 60 and 95% alcohol, or 60 and 95% acetone. The fatty fractions removed by the solvents all proved completely inactive.

Vaccine Virus. The testicles of rabbits which had been inoculated 4 days previously with vaccine virus were removed, dried, and prepared as described above. This material, after shaking for 6 days with alcohol, acetone, chloroform or toluol, gave typical vaccinal lesions in rabbits when injected intradermally. The virus treated with chloroform and alcohol was less active than that treated with acetone or toluol. The fatty fractions removed by the solvents were inactive.

Herpes Febrilis Virus. Fresh rabbit brain containing active *Herpes febrilis* virus was treated in the manner described. Some of the fresh brain was put in 40% glycerol as a control and some of the untreated desiccated brain was also kept as a control. After extraction with dry acetone, toluol, chloroform, or alcohol, the various extracts and residues were taken up in Locke's solution and 0.25 cc. of each was injected into the brains of young rabbits. The rabbit inoculated with the glycerol control material died in 3 days, but its brain had undergone too much post-mortem change for a study of the nuclear changes due to the virus. The desiccated material produced typical *Herpes febrilis* reaction, killing the animal in 5 days. The brain showed very many typical eosin-staining nuclear inclusions. The other rabbits injected with extracts or residues from the various solvents were not affected in any way. All surviving rabbits were tested 6 weeks later by reinoculation of active herpes virus with proper controls and all died very promptly, showing that no immunity had been produced by the previous inoculation of desiccated virus treated with the various organic solvents.

Summary: The causative agent of Chicken Tumor I resists freezing and desiccation, and this dried material retains its activity after being shaken for 6 days in dry acetone, alcohol, and toluol. The

same is true of vaccine virus. The dried material of Chicken Tumor I is rendered inactive by shaking with chloroform while vaccine virus in the dried state withstands such treatment. *Herpes febrilis* in brain tissue resists freezing and desiccation but is destroyed when the dried material containing it is shaken with alcohol, acetone, toluol, or chloroform. None of the agents studied are extracted in their active form from the dried material by the solvents.

From these results it would appear to be impossible to distinguish between the agent of Chicken Tumor I and viruses by desiccation and subsequent treatment with the ordinary organic fat solvents.

4357

Reaction Between Proteins and Diazotized Aromatic Amines in Neutral Solution.*

MICHAEL HEIDELBERGER AND FORREST E. KENDALL.

From the Laboratories of the Department of Practice of Medicine, Presbyterian Hospital and College of Physicians and Surgeons, New York City.

In a series of 3 challenging papers entitled "Chemospecific Antigens" Klopstock and Selter[1] have raised a number of important questions dealing with the chemical basis of certain immune reactions. While several of their minor points will be dealt with in connection with other work now in progress, the present note concerns the underlying thought and principal conclusion of their work, namely, that no chemical interaction takes place in neutral solutions of diazotized aromatic amines and proteins or lipoids. It is held that such solutions are merely simple mixtures, and that "eine chemisch zu verstehende Substitution des Chemikals im Eiweissmolekül bei der chemospezifischen Komplexantigenbildung keine Rolle spielt." Klopstock and Selter have failed entirely to report chemical control experiments in testing this conclusion, and the following are therefore submitted:

I. Conditions the same as in Paper II, p. 455, showing appearance of chemospecific antigen only after mixtures had stood in the cold 1 to 24 hours.

1% diazotized arsanilic acid, "neutralized to litmus."

---

* The work reported in this paper was made possible by the Harkness Research Fund of the Presbyterian Hospital.

[1] *Z. f. Immunitätsforschung*, 1928, lv, 118, 450; lvii, 174.

2 vols. rabbit serum (K. and S. used beef serum in this experiment, rabbit serum in others).

Dubosc Colorimeter* Readings

Immediately after mixing			1 hour in ice-box after mixing		
	1 vol. diazo solution, 2 vols. H_2O	Diazo-serum mixture		1 diazo, 2 H_2O	Diazo-serum mixture now orange in color
	4	1.4		6.3	1.1
5 min. later at room temp.	4	0.8	10 min. later at room temp.	6.3	1.1
13 min. later at room temp.	4	0.4			

* Thanks are due Prof. H. T. Clarke for suggesting the greater precision obtainable with the colorimeter.

Since color formation could conceivably have been due to some other serum component than protein, a solution of 3-times recrystallized, dialyzed egg albumen (containing 0.018 g. per cc.) was next used.

II. No difference to the eye immediately after mixing.

Dubosc Colorimeter Readings after 1 hour in ice-box

1 vol. diazo solution, 2 H_2O	1 diazo solution, 2 egg albumen
2.3	1.7

Since the neutral diazo arsanilic acid solution darkened rapidly, as noted by Klopstock and Selter, making readings after several hours unsatisfactory, diazotized sulfanilic acid was substituted. This darkens comparatively little on standing in the cold, and, in addition, antisera to sulfanilic-azo-proteins were available. Moreover, since "neutrality to litmus" is a vague term an experiment was run at pH 6.8, but since it might be objected that this is toward the alkaline side of "neutral to litmus" and that actual coupling took place for this reason,† there are given below only the results at pH 6.3-6.4, at which blue litmus is faintly reddened.

III. Dialyzed egg albumen solution, 0.022 g. per cc., pH 6.3.
0.75% solution of diazotized sulfanilic acid, pH 6.4.

---

† This affords the simplest explanation of the greater tendency of Klopstock and Selter's "serum-control preparations" to yield chemospecific antigens. After being subjected to alkali and acid the products were dissolved "bei alkalischer Reaktion." (Paper II. p. 452.)

Klett Colorimeter Readings

A. After 6 min. in ice-water a faint yellow color appeared only in the albumen-diazo solutions.		
10 cc. H_2O 1 cc. diazo solution		10 cc. H_2O 10 cc. egg albumen solution 2 cc. diazo solution
1 min. after removal	40	16.2
18 ” ” ”	40	7.0
B. After 2.5 hours in the ice-box the albumen-diazo solutions were bright yellow, the controls almost colorless.		
0.5 min. after removal	40	6.6
6.5 ” ” ”	40	6.1
C. After 23 hours in the ice-box the albumen-diazo solutions were deep yellow, the controls pale yellow.		
	45	7.8

Tubes set up with protein and M/15 phosphate buffer at pH 6.4 instead of water showed approximately twice as much dye formation.

Serum tests with diazo-albumen solution III C (above)

Diazo-albumen Solution	Anti-Egg Albumen Rabbit Serum, 1:1	Anti-Sulfanilic-Azo-Egg-Albumen-Dye Rabbit Serum absorbed with Egg Albumen, 1:1	0.5 cc. of sera and dilutions 2 hrs. at 37°, over night in ice-box
1:10		+±	Serum and solution controls negative
1:25		++	
1:100		+	
1:200	++	—	
1:500	+++	—	

It is thus evident that true chemical interaction, with resulting dye formation, takes place in neutral mixtures of diazotized aromatic amines and proteins. In neutral solution it is of course impossible to obtain other than mixtures containing much unchanged protein, unchanged diazo compound, and a little protein dye, but if a buffer or if alkali is present to neutralize the acid liberated, the reaction goes in the direction of complete dye formation. Enough colored compound is formed, however, even in faintly acid solution to be observed readily and to be detectable by the specific precipitin test. Landsteiner's conception of the reaction[2] is therefore in accord with chemical data, while Klopstock and Selter's main thesis in the series of papers under discussion is utterly without experimental basis.

---

[2] *J. Exp. Med.*, 1924, xxxix, 631; *Klin. Wchschr.*, 1927, vi, No. 3.

4358

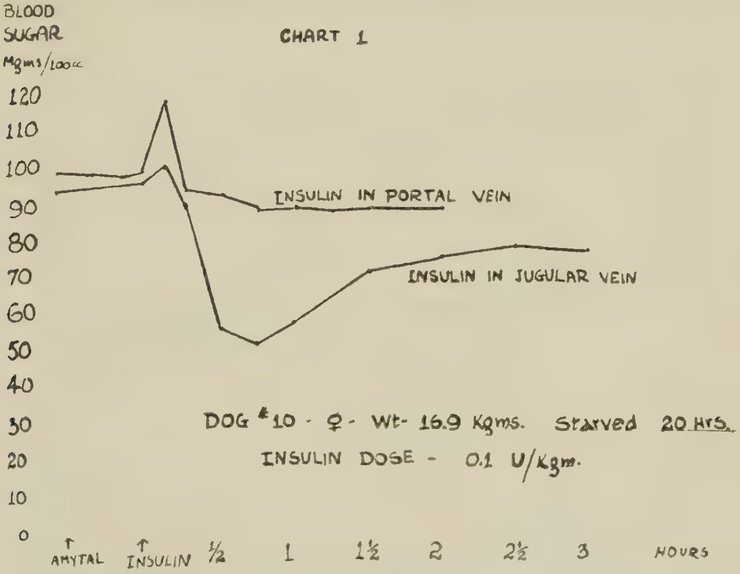
Hyperglycemia Following the Portal Injection of Insulin.

WILLIAM S. COLLENS* AND JOHN R. MURLIN.

From the Department of Vital Economics, University of Rochester Medical School.

Experimental and clinical reports have appeared in the literature to indicate that the liver plays a part in regulating the activity of insulin. If it were conclusively proven to be so, then diabetes mellitus might be considered in part as a result of a disturbance of this regulatory mechanism of the liver.

Arborelius and Akerren[1] injected insulin into the serous coat of the intestine of rabbits and compared the drop in blood sugar with that which occurred following subcutaneous injections. They observed that hypoglycemia did not occur as readily following subserous injections and inferred that the liver had an inhibiting influence upon insulin. Brugsch and Horsters[2] noted a decrease in the potency of insulin, when insulin was incubated with liver pulp suspensions. Loewi,[3] although much questioned, claims to have iso-



---

* Assistant, Research Medicine, Harry Caplin Pediatric Research Laboratory, Jewish Hospital of Brooklyn, N. Y.

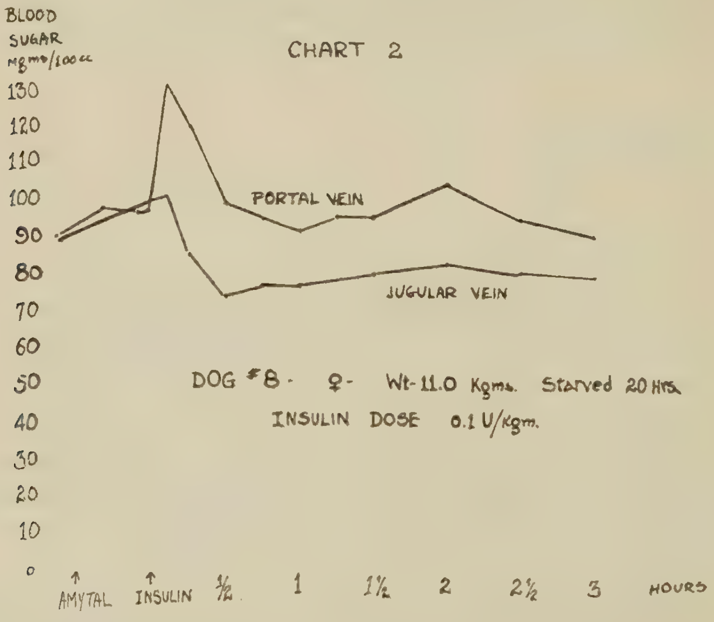
[1] Arborelius, M., and Akerren, Y., *Skand. Arch. f. Physiol.*, 1927, l, 35.

[2] Brugsch, T., and Horsters, H., *Biochem. Z.*, 1927, clxxxviii, 147.

[3] Loewi, O., *Klin. Woch.*, 1927, vi, 137.

lated a hormone from the liver which is antagonistic to insulin, particularly as regards its effect on the uptake of glucose by the red blood corpuscles. He calls this substance glykamin. His premise that insulin causes a fixation of sugar in the red blood corpuscles, was not confirmed by Harpuder.[4] Shope[5] studied the distribution of sugar between the corpuscles and plasma of normal and diabetic bloods. He found an equal amount of sugar in the plasma and the cells of all cases and observed no significant alteration in its distribution following insulin therapy. Zuelzer[6] also questions the nature of this hormone and suggests that it must first be proven that it is not adrenalin. A transient initial hyperglycemia following the subcutaneous injection of insulin has been observed by Guardabassi[7] in normal dogs and by Rosello[8] in a diabetic dog.

This study was undertaken to determine, by the direct introduction of insulin into the portal circulation, whether the liver plays any part in regulating insulin activity. Dogs which had been previously starved for 24 hours were used in all the experiments. Amy-



---

[4] Harpuder, K., *Klin. Woch.*, 1928, vii, 266.

[5] Shope, R. E., *J. Biol. Chem.*, 1928, xxviii, 111.

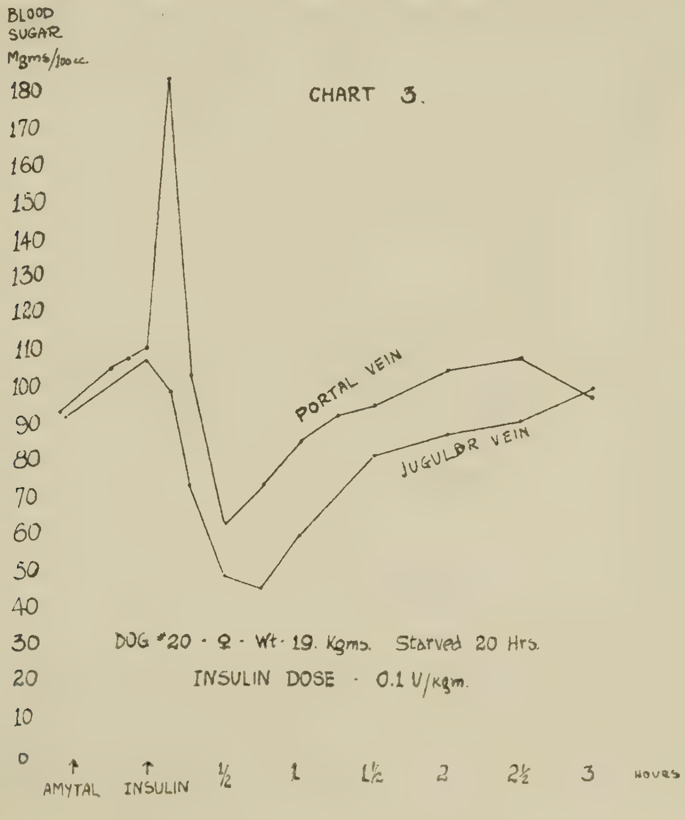
[6] Zuelzer, G., *Klin. Woch.*, 1928, vii, 312.

[7] Guardabassi, M., *Ber. Ges. Physiol. Exp. Pharm.*, 1927, xli, 86.

[8] Rossello, H., Benatti, D., Balea, S. E., *Ann. de la Facultad de Med.*, 1926, xi, 259.

tal was the anesthetic used because it has no influence on the blood sugar. The Folin Wu method was employed for the blood sugar determinations. Each experiment consisted of the alternate injection of the same amount of insulin into the portal or jugular vein at weekly intervals. The volume of solution of insulin injected was never greater than 1 cc. Insulin was made up in a solution of HCl at a pH of approximately 3.0. A large stock solution of insulin obtained from the Eli Lilly Co. was prepared and aliquots of the same solution were used in all the experiments.

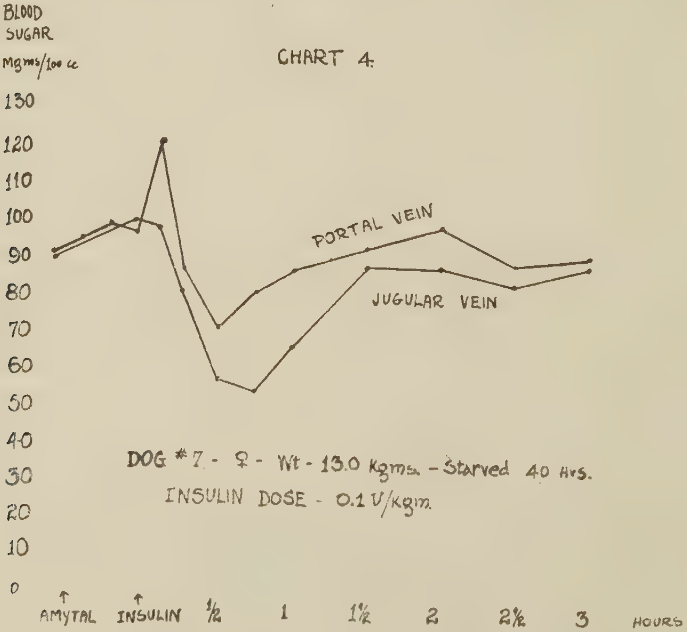
Results: Five experiments were performed. The constancy of the results obtained may be seen from Charts I to V. It will be noted that in every experiment, the portal injection of insulin results in an immediate sharp *rise* in the blood sugar of 20 to 80 mgm. This rise occurred within 5 minutes after the injection of the insulin and was then followed by a rapid decline. No such rise occurred following the systemic injection of the same dose of insulin. In order to rule out the possibility that the rise in blood sugar resulted from the mechanical effect of the injection, or the slightly acid state of the



solution in which the insulin was kept, saline and HCl (pH 3.0) were injected into the portal vein but no effect upon blood sugar was noted (Chart VI).

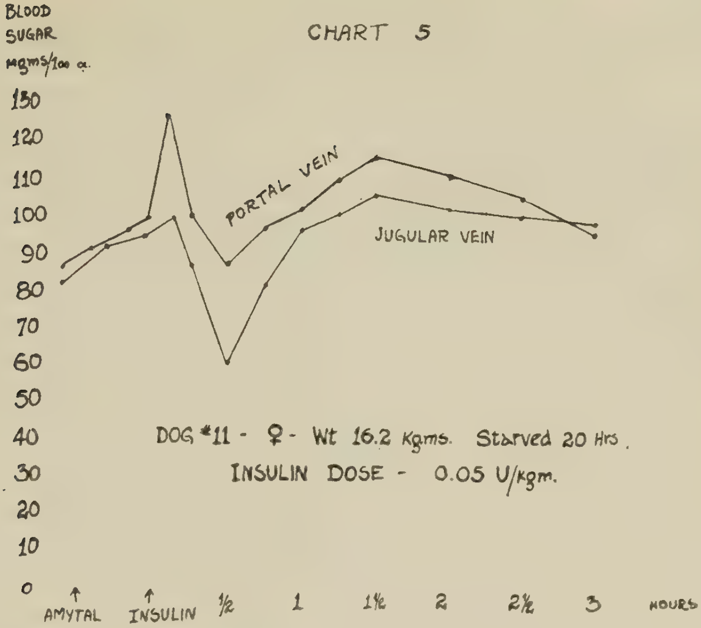
As an additional control, the blood sugar was studied 5 minutes after injection and over a 5 hour period following the intraperitoneal injection of amytal and it was observed to remain at a normal stationary level. Mechanical manipulation of the liver was avoided, for this alone was found to cause a rise in the blood sugar.

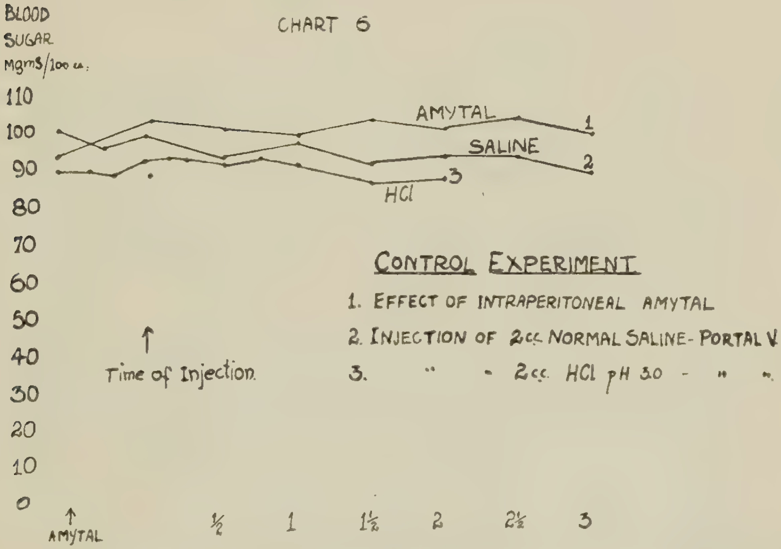
Discussion: It is quite obvious that the portal injection of insulin produces a distinct temporary rise in the blood sugar. This phenomenon permits of only 2 explanations; either that the insulin causes the discharge of an anti-insulin substance in the liver which is antagonistic to its action, or the sudden introduction of insulin into the liver causes a rapid glycogenolysis. The latter explanation is at present regarded as the more probable one—first because of the transient nature of the phenomenon, and second because it has been demonstrated that large doses of insulin even when injected systemically not only lower the blood sugar, but reduce the glycogen deposits in the liver. McCormick and Macleod[9] gave subconvulsive doses of insulin to normal rabbits and they observed an average glycogen content in the liver of 2.9% as compared with 4.1% glyco-



---

[9] McCormick, N. A., and Macleod, J. J. R., *Trans. Royal Soc. Canada*, Sec. V., 1923, xvii, 63.





gen in control animals. Dudley and Marrian[10] found 5.5% liver glycogen in normal rabbits and only 1.9% in insulinized rabbits. Bodo and Marks[11] observed, in perfusion studies of the isolated mammalian liver, that added insulin causes a breakdown of glycogen. The glycogenolytic properties of insulin have also been observed by Brugsch,[12] Nitzescu[13] and Gigon and Staub.[14] Macleod[15] believes that glycogenolysis is the most important single factor in restoring the blood sugar during the hypoglycemic state. Our experiments, by the direct introduction of insulin into the portal circulation, lend further confirmatory evidence to the conception that insulin in contact with liver cells, is an active glycogenolytic principle.

---

[10] Dudley, H. W., and Marrian, G. F., *Biochem. J.*, 1923, xvii, 435.

[11] Bodo, R., and Marks, H. P., *J. Physiol.*, 1928, lxv, 48.

Conclusion: A transient hyperglycemia follows the portal injection of insulin.

We wish to express our appreciation to Mrs. Gladys Havens for her technical assistance in the work.

4359

The Source of Fibrinogen.

P. D. MC MASTER AND D. R. DRURY.

From the Laboratories of The Rockefeller Institute for Medical Research.

The formation of fibrinogen has been ascribed to many tissues and organs of the body. Whipple,[1] Goodpasture[2] and others suggested that this substance takes its origin from the liver and intestinal tract; and recently Foster and Whipple[3] have presented excellent evidence that the liver is the main if not the sole source of fibrinogen. Schultz, Nicholes and Schaefer[4] have produced corroboratory data. Final proof in the matter has waited, however, upon the findings in liverless animals.

In 4 hepatectomized rabbits[5] we have determined the concentration of blood fibrinogen before operation and at various periods thereafter. A postoperative decrease in the substance was always found. In 6 others a partial depletion of fibrinogen was accomplished after hepatectomy and its concentration in the blood was followed until death. Controls subjected only to defibrination were also studied.

---

[12] Brugsch, T., Benatti, A., Horsters, H., and Katz, R., *Biochem. Z.*, 1924, cxlvii, 117.

[13] Nitzescu, L. I., and Popescu-Inotesti, C., *Compt. Rend. Soc. Biol.*, 1923, lxxxix, 1403.

[14] Gigon, A., and Staub, *Klin. Woch.*, 1923, ii, 1670.

[15] Macleod, J. J. R., "Carbohydrate Metabolism and Insulin," Longmans, Green and Co., 1926, 171.

[1] Whipple, G. H., *Am. J. Physiol.*, 1914, xxxiii, 50.

[2] Goodpasture, E. W., *Am. J. Physiol.*, 1914, xxxiii, 70.

[3] Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lviii, 407.

[4] Schultz, E. W., Nicholes, J. K., and Shaefer, J. H., *Am. J. Path.*, 1925, i, 101.

[5] Drury, D. R., *J. Exp. Med.*, 1929, in press.

The initial defibrination was accomplished under ether anesthesia by slowly injecting into the jugular vein 100-150 cc. of compatible defibrinated blood while removing an identical quantity from the carotid artery. The procedure was repeated 4 or 5 times. This reduced the blood fibrin concentration to one third to one fifth its original amount. By continuing the blood exchange a still greater reduction was sometimes brought about in the controls. Fibrinogen was estimated in the form of fibrin by the method of Foster and Whipple[6] as modified by Schultz, Nicholes and Schaefer.[4]

In the controls fibrinogen regeneration was exceedingly rapid. Within 5 or 6 hours after a 90% reduction, a complete return to the previous amount was observed, regeneration far more rapid than has been reported for the dog.[7] Twenty-four hours later the concentration had reached a level 50% above the normal figure and in two instances it had doubled within 48 hours.

The findings were wholly different in the liverless rabbit. In 6 animals blood fibrin determinations were made immediately after the hepatectomy and then the partial defibrination was carried out, reducing the circulating amount by 65 to 80%. Four or 5 hours later a slight rise, about 10%, was usually but not always found, indicating the existence of a small reserve within the tissues. Thereafter, however, a marked and progressive decrease from the previous very low figures was invariably observed. Blood specimens taken 9 to 18 hours later showed 38 to 74% reduction in blood fibrin. No changes in the blood hematocrits were found to account for either alteration.

In the hepatectomized rabbits in which no defibrination was attempted there was a similar marked progressive decrease in blood fibrin. In 4 instances the blood fibrin concentration had fallen, 24 hours after hepatectomy, to 35-45% of its preoperative level.

The findings prove that fibrin regeneration does not occur in the absence of the liver.

---

[6] Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lviii, 365.

[7] Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lviii, 393.

4360

The "Stained Slide" Microscopic Agglutination Test: Application to (1) Rapid Typing of Pneumococci; (2) Determination of Antibody.

ALBERT B. SABIN. (Introduced by William H. Park.)

From the Littauer Pneumonia Research Fund of New York University and the Department of Bacteriology of the New York University Medical School.

The importance of obtaining an early diagnosis of the type of infecting pneumococcus, especially in cases of lobar pneumonia to be treated with serum, has resulted in the development of many methods. The microscopic agglutination test just described, since it required very few organisms, was expected to reduce the time for a determination of type. Attempts to type the organisms directly from the sputum were unsuccessful. The fact that in the microscopic test the sputum was exposed to the immune serum for a very short time only, was at first thought to be responsible for the negative results. Small amounts of sputum were accordingly mixed with varying amounts of serum, and incubated in sealed capillary tubes at 37° C. for different periods of time; these mixtures were subsequently smeared and stained. The unsuccessful results are due possibly to the presence of some interfering factor or unfavorable physical condition in the substance in which the pneumococci are coughed up. Most of the sputa injected into the mouse's peritoneum are digested within 2 hours, and in 3-4 hours, sufficient organisms may be found to carry out a microscopic typing test. Broth may be used as a culture medium in the absence of mice, but the mouse is preferred because its peritoneum is somewhat selective for pneumococci, and because results can be obtained in a shorter time.

The procedure for microscopic typing is as follows: One cc. of a fresh sample of sputum is injected intraperitoneally into a mouse. 3-4 hours after injection some of the peritoneal fluid is obtained by capillary puncture. A glass slide is marked off into 4 parts, and a minute drop of the peritoneal fluid is expelled onto each one of the 4 partitions. The first is smeared with saline for control, and the others with a loopful of a 1-10 dilution of type I, of type II, and of type III, diagnostic serums respectively. This dilution of serum is chosen to largely eliminate group agglutinins. The smears are made thin, so that they dry rapidly; they are then stained for one-half minute with a fuchsin solution (10 cc. saturated alcoholic solution of basic fuchsin plus 90 cc. distilled water). The stain is washed off in running water, and the smears are examined with the oil-immersion lens. If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of the corresponding type. If no reaction occurs in any of the smears, and pneumococci are clearly seen, a diagnosis of group IV is made. When it is desired to know whether the organism is one of the fixed types of Group IV, a similar procedure is carried out with the corresponding diagnostic sera. Naturally occurring clumps of organisms differ in appearance from those produced by specific agglutination; they can be recognized by their occurrence in the saline control smear as well. Unless a fresh sample of sputum is used many of the organisms will have undergone autolysis and therefore more time must be allowed for growth. Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse the type may be confirmed. In the case of type III sufficient organisms for a microscopic typing are present as early as 2 hours after injection. The appearance of the specific reaction with type III, differs from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule. More than 100 cases have already been typed by this method at the Harlem Hospital with perfect agreement as compared with the results obtained by the older methods.

Microscopic Antibody Test. It has been suggested[1] that the dosage of antipneumococcus sera be controlled by an antibody test on patient's blood. Taking advantage of the fact that the microscopic agglutination test requires only minute amounts of serum, it was performed on patients who had just recovered from lobar pneumonia or whose blood had been rendered anti-bacterial by therapeutic administration. Positive tests were obtained only against the homologous type of pneumococcus. The technique for carrying out the microscopic antibody test is as follows:

A drop or more of the patient's blood is taken with a capillary. This is either centrifuged or after coagulation and synaeresis, a minute amount of serum is smeared with a loopful of a heat-killed, saline suspension of the type of pneumococcus for which it is desired to determine agglutinins. The drops are thoroughly mixed and smeared very thin, allowed to dry in air, and stained for one-half minute with the fuchsin solution recommended for the microscopic typing test. The specific agglutination reaction is obtained here, but the clumps tend to be smaller. The sensitivity of this method was compared with the rapid macroscopic method of Arlyle

---

[1] Park and Cooper, *Trans. Sect. on Path. and Physiol., Am. Med. Assn.*, 1927.

Noble[2] and the regular macroscopic tube agglutination, and it was found to be 2½-3 times as sensitive as either of the other methods when a standard diagnostic serum was used for the comparison. Observations which aim to establish with the aid of the microscopic antibody test a more exact guide for the administration of anti-pneumococcus sera are being collected at Harlem Hospital.

The microscopic agglutination reaction has also been tried successfully with *B. typhosus* and *meningococcus*. Probably it might be applied as well wherever the older methods of demonstrating agglutination are used, and more advantageously in many instances where, without loss of time, the older methods are either unsatisfactory or impossible.

I wish to thank Dr. William H. Park and Dr. Jesse G. M. Bullowa for their kind cooperation and numerous valuable suggestions.

4361

A Method of Studying Virus Infection and Virus Immunity in Tissue Cultures.

T. M. RIVERS, E. HAAGEN AND R. S. MUCKENFUSS.

From the Hospital of The Rockefeller Institute for Medical Research, New York.

The fact that certain viruses, particularly vaccine virus, can be cultivated *in vitro* in the presence of susceptible living tissue is now well established. For the cultivation of viruses, small pieces of corneal or testicular tissue from the rabbit, or finely minced chick embryos have usually been employed. In only a few instances[1] has the claim been made that "inclusion bodies" were observed in tissues infected and cultivated *in vitro*. These claims, however, have not been sufficiently corroborated to be accepted generally.

The inclusions referred to above are characteristic of some diseases, *e. g.*, *vaccinia* and *herpes febrilis*, and, regardless of their nature, represent the visible intracellular changes incident to or resultant from infection or injury of cells by certain viruses. Therefore, it seemed likely that, if it were possible to develop a method by which susceptible tissues infected and cultivated *in vitro* regularly evidence these characteristic intracellular alterations, knowledge concerning infection and immunity might be obtained through the proper use of various combinations of viruses with normal tissue, immune tissue, plasma from normal animals, plasma from immune animals, normal serum, and immune serum. The purpose of this paper is to describe briefly a method by means of which such studies may be conducted.

---

[2] Noble, A., *J. Bact.*, 1927, xiv, 287.

[1] Gins, H. A., *Zt. Hyg.*, 1916, lxxxii, 89.

Inasmuch as the rabbit is susceptible to vaccine virus and also to *herpes febrilis* virus, and since the former agent produces cytoplasmic inclusions while the latter induces nuclear changes, rabbit tissue, vaccine virus, and herpetic virus were chosen for the work. Corneal epithelium was selected for the study, because in this tissue the intracellular changes caused by the viruses have been extensively investigated. Furthermore, the work of Aldershoff and Broers[2] indicated that the use of cornea *in vitro* would be attended by success.

The following is the technique employed. A rabbit is exsanguinated for plasma. Then the spleen is excised, from which a tissue extract is prepared. Finally, both eyes, intact, are enucleated and thoroughly washed in Ringer's solution to remove as many contaminating bacteria as is possible. With a sterile cataract knife the control eye is cross-hatched with closely spaced scarifications just deep enough to penetrate the epithelial layer. The test eye is treated in a similar manner with the exception that between each scarification the knife is dipped in a virus emulsion. The cornea is then removed from each eye and divided into 4 to 8 pieces. The pieces from the test eye are further inoculated by placing them for one hour in an emulsion of virus. In the bottom of a 50 cc. pyrex centrifuge tube a clot (3 cc.) of plasma and spleen extract is made. On top of this clot is placed one piece of normal or infected cornea which is then covered by another clot of plasma and tissue extract. The tubes, sealed with sterile corks, are placed in an incubator at 37° C. for 24 to 48 hours. Then the clots, containing their bits of cornea are carefully removed from the tubes and fixed in Zenker's fluid. Sections are stained with eosin and methylene blue or according to the method of Giemsa. In this work, a potent virus, freshly prepared and containing no preservative, must be used.

Many of the cultures made in the manner described above were free from bacteria, others contained a few colonies that did not interfere with the action of the viruses, while still others were sufficiently contaminated to render them valueless. In properly prepared cultures, abundant typical Guarnieri bodies appeared in the cytoplasm of corneal epithelial cells infected with vaccine virus, and numerous acidophilic inclusions occurred in the nuclei of cells injured by herpetic virus. Neither type of inclusion, however, was found in control pieces of cornea. In infected and uninfected bits of cornea, definite evidence of growth of epithelial cells was frequently seen. The results of this work, including observations obtained through the use of tissues and plasma from immune animals, will be described in detail in a later communication.

---

[2] *Anns. Inst. Pasteur*, 1906, xx, 779.

4362

Respiratory Quotient of the Brain.

H. E. HIMWICH AND L. H. NAHUM.

From the Department of Physiology, Yale University School of Medicine.

We have studied the respiratory quotient of the brain of dogs by analysis of the blood entering and leaving that organ. Under amytal anesthesia, except for one animal, the superior longitudinal sinus of large female dogs was exposed and a sample of venous blood was drawn from it simultaneously with one of arterial blood from the femoral artery. The blood samples were analyzed for carbon diox-

TABLE I.
Respiratory Quotients of Intact Animal and of Brain.

Condition of Animal	Air R.Q. of Intact Animal	Blood R.Q. of Brain
Fasted 2 days	0.75	1.03 1.01
Fasted 2 days. No anesthetic.	0.77	1.06 0.97
Phlorizinized	0.69	0.97 1.02
Depancreatized	0.67	0.94 1.00
Depancreatized and Phlorizinized	0.69	0.96 1.00 1.00 0.99

TABLE II.
Respiratory Quotients of Brain.

No. of Animals	Condition of Animal	Number of Observations	Average R.Q.
4	Normal	7	0.99
1	No anesthesia	4	1.04
4	Phlorizinized	12	1.00
4	Depancreatized	11	0.99
1	Depancreatized and Phlorizinized	4	0.99
—		—	—
14		38	1.00

ide and oxygen by the method of Van Slyke and Neill. The respiratory quotient of the intact animal was determined on samples of expired air collected before the blood was drawn.

In Table I are presented typical observations of a group which are summarized in Table II. 38 observations of the brain were made in 14 dogs and the average of the respiratory quotient was 1.00.

4363

The Meaning of Bier's Spots.

PEYTON ROUS AND H. P. GILDING.

From the Rockefeller Institute for Medical Research, New York City.

By vital staining with highly diffusible dyes we have found that sudden reductions of the blood volume lead to readjustments whereby the organs essential to the maintenance of life and to recovery continue to be well served by the circulation but at the expense of unessential ones, both visceral and peripheral.[1] The animals studied were white cats, rabbits and rats. The heart and lungs, the alimentary tract throughout its length (inclusive of the tongue and the gall bladder), the liver, pancreas, red bone marrow, and the respiratory muscles suffer practically no deprivation, even when the depletion has been pushed so far as to endanger life; whereas the skin and the voluntary muscles in general, the omentum, fat depots and urinary bladder are greatly neglected. One would expect the effective circulation through the spleen to cease, as does indeed happen. All the other neglected organs develop an ischemic patching except the bladder, which suffers as a whole. That in the skin resembles the so-called Bier's spots, blanchings noted in engorged human skin deprived of circulation. Our further work indicates that it is identical therewith.

Bier's spots appear gradually and enlarge by peripheral extension and coalescence. The ischemic patching of animals also develops gradually in skin from which the blood supply has been not quite all cut off; and the patches appear and spread as do Bier's spots. These latter are pallid, irregular, sharply outlined blotches on a congested skin, while the ischemic patches occur as similar areas devoid of stain, though scattered through a tissue which becomes well colored despite the reduced circulation. These differences are incidental to the differing methods of demonstration. Bier's spots are far more frequent where gravity or other influences act to empty the vessels *in stasis* than where they remain full. The patching in animals is also favored by these influences. Bier's blanchings develop far more frequently in the "red spots" which are still fed by a trickle of arterial blood from the marrow collaterals than where the skin is violet with stagnant blood. The combination of nearly empty vessels and a slight, inadequate blood flow is precisely that which leads to the ischemic patching of animals.

---

[1] Reported at the Association of American Physicians, May, 1928.

Bier's spots tend to recur where they appeared previously, and so too with the ischemic patching. The various local influences above mentioned go far to explain this for both. Those spots which become most completely blanched often persist for a brief period after the circulation is restored to the arm.[2] The ischemic patching tends to last longer, sometimes for a half hour or more, after the blood volume has been made up and the original blood pressure restored to the previous level or a higher one. In man the occlusion which gives rise to the spottings can be maintained but for a short while, and hence evidently their failure to endure.

The blanching in man is caused by a contraction strong enough to drive blood out of the small vessels even when the intravascular pressure has been so greatly raised in the region studied that punctate ecchymosis is occurring. The contractile impulse dominates over that which gives rise to vascular dilatation next cutaneous abrasions, fresh and old, the purple of the injured skin being replaced by pallor. On the other hand it is not effectual against histamine dilatation.

There can be no doubt that the cells involved in Bier's spots are abnormally irritable. Tapping the congested skin, or a light stroking which would elicit no response normally, causes a pronounced local reaction of dilatation or contraction when the cutaneous circulation has been cut off for some minutes. Usually the response is contractile, white patches appearing. Often they are situated at a distance from the point stimulated.

The slowly developing vascular contraction which is responsible for Bier's spots and for the ischemic patching of animals acts to narrow the circulatory bed when the blood volume has been reduced and thus tends to conserve the flow to vital organs.

---

[2] Lewis, T., ''The Blood Vessels of the Human Skin and Their Responses,'' Shaw and Sons, Ltd., London, 1927.

4364

Study of Bacteria Found in Eighty Cases of Non-Tuberculous Infections of the Urinary Tract.

M. F. GOURLEY. (Introduced by H. J. Sears.)

From the Department of Bacteriology and Hygiene of the University of Oregon Medical School.

A single bacteriological examination of a catheterized specimen was made in each of 80 cases of urinary tract infection consisting chiefly of pyelitis. Only 3 cases failed to yield positive cultures. From the remaining 77 cases a total of 90 cultures was isolated. In 63 cases one organism only was found. In 12, two were present, and in 1, three different species were obtained. Among the 90 cultures 13 different species were represented as follows:

	Frequency	%
Staphylococcus aureus	19	21.1
Staphylococcus albus	18	20.0
B. coli	29	32.0
B. pyocyaneus	6	6.6
Streptococcus hemolyticus	3	3.3
Streptococcus viridans	3	3.3
B. alcaligenes	4	4.4
Non-hemolytic streptococcus	3	3.3
Enterococcus	1	1.1
B. pseudotetanicus	1	1.1
B. proteus	1	1.1
M. catarrhalis	1	1.1

It is interesting to note from the above data that the staphylococci were the most numerous group found and that the total Gram negative cocci outnumber the total Gram negative bacilli. The former represented 51.1% of the total, and the latter 46.6%.

That only 69% of the Gram negative bacillus group proved to be *B. coli* shows that the error in making diagnosis purely on the basis of a Gram stain of the urinary sediment may be very large.

A detailed cultural study of all the organisms was made, but it seems pertinent to mention here only certain results of this study.

A study of the hemolysing properties of the cultures revealed 1 hemolytic *B. coli*, 6 hemolytic *Staphylococcus aureus* and 2 hemolytic *Staphylococcus albus*.

Dudgeon[1] has called attention to a group of atypical colon bacilli that are characterized by their slow fermentation of lactose. In the above series 2 such cultures were encountered. Both belonged to the *B. coli communis* group and showed the presence of gas on lactose only after 4 days incubation.

All the *B. pyocyaneus* cultures were typical regarding their production of pigment and the liquefaction of gelatine. One of the 6 strains, however, deviated from the usual behavior of this organism in its acid production from the sugars. Glucose, lactose, and maltose were rapidly attacked by this strain, the Andrade's indicator of the medium showing a strong acid reaction in 24 hours. This is the first lactose fermenting *B. pyocyaneus* ever encountered in this laboratory. Sherwood[2] mentions one such strain.

B. pseudotetanicus is a large Gram negative aerobic bacillus with sub-terminal spores. It is generally credited with being of soil origin. The only other reference to this organism as a urinary tract infectant is in the paper of Caldwell[3] who found it 12 times in 112 cultures of Gram negative bacilli. In a subsequent examination of this case this organism was not found.

4365

Ephedrine-Epinephrine Antagonism.

C. H. THIENES.

From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

Antagonism of epinephrine action by high concentrations of ephedrine (1:9000-1:6000) has been described by several European workers,[1] using Magnus preparations of excised small intestine of rabbit and cat and of excised guinea pig uterus. In extending the study of the ephedrine-epinephrine antagonism to Magnus strip preparations of small intestine, colon and uterus of various species (cat, rabbit, dog, rat, guinea pig) I have found that it occurs with all these organs. Furthermore, not only did high concentrations of ephedrine antagonize epinephrine inhibition, but low concentrations as well (1:100,000-1:25,000), and often to a marked degree. Segments of uterus of the rabbit and of the pregnant cat, treated with ergot alkaloids, to produce an inhibitory response to epinephrine, exhibited the antagonism as completely as did the organs whose normal response to epinephrine is one of inhibition or relaxation. The "depressant" action of epinephrine (1:50,000,000-1:1,000,000) on all these organs was opposed by ephedrine, whether applied to the tissues before, or a few seconds after, the application of epinephrine.

---

[1] Dudgeon, L. S., and Pulvertoft, R. J. V., *J. Hyg.*, 1927, xxv, 285.

[2] Sherwood, N. D., Johnson, T. L., and Radotincky, I., *Univ. Kansas Sci. Bull.*, 1926, xvi, 91.

[3] Caldwell, J. A., *J. Inf. Dis.*, 1928, xliii, 353.

[1] Nagel, A., *Arch. exp. Path. Pharm.*, 1925, cx, 128. Kreitmair, H., *Arch. exp. Path. Pharm.*, 1927, cxx, 189. Reinitz, N., *Compt. rend. soc. biol.*, 1928, xcviii, 809.

"Depression" of segments of rabbit duodenum by a mixture of epinephrine and ephedrine occurred, although the same concentrations applied separately and in sequence, exhibited the usual antagonism. This indicates that there is no chemical action between the 2 drugs outside the tissues. Antagonism of epinephrine "depression" by ephedrine occurred whether ephedrine itself caused contraction, relaxation or no demonstrable effect on the activity of the muscle. This fact, and the fact that low concentrations as well as high concentrations of ephedrine were effective in antagonizing epinephrine do not support the opinion of Nagel[1] that the antagonism is due to a muscle stimulating action of ephedrine. It would seem rather that it is due to some as yet ill-defined action of ephedrine on the sympathetic nerve-muscle connections.

4366

Action of Ergot Alkaloids on Intestine and Uterus.

C. H. THIENES.

From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

That ergot alkaloids abolish the muscle contracting action of epinephrine has been known for many years.[1] It was only recently that an effect of the alkaloids was demonstrated on the inhibitory action of epinephrine[2] and proposed as a means of bio-assay of ergot preparations[3] by European workers. More recently still, Mendez[4] reported inability to confirm the findings of previous workers. My results will therefore be of interest, in that they confirm the reports of the European investigators that the intestinal sympathetics can be markedly depressed, if not paralyzed by ergot alkaloids.

---

[1] Dale, H. H., *J. Physiol.*, 1906, xxxiv, 163.

[2] Planelles, *Arch. exp. Path. Pharm.*, 1924-5, cv, 38.

[3] Issekutz, B. von, and Leinzinger, M. von, *Arch. exp. Path. Pharm.*, 1928, cxxviii, 165.

The ordinary Magnus strip method, using Tyrode's and Locke's solutions and segments of small intestine of rabbit and cat, and of colon of rabbit, cat and guinea pig, and uterus of rabbit, was employed. Ergotamine tartrate (Sandoz)* and Adrenaline-HCl tablets (Parke-Davis) were the preparations used, the ergotamine in concentrations of 1:250,000 to 1:500,000 and epinephrine in concentrations of 1:10,000,000 to 1:750,000. In all trials upon the small intestine of the rabbit and cat, epinephrine caused relaxation or inhibition before the application of ergotamine, but following ergotamine the action of epinephrine was to a large extent or entirely abolished, as shown in the figure. However, concentrations of ergotamine up to 1:20,000 failed to influence the action of epinephrine upon the colon of the rabbit, cat or guinea pig; it was also impossible to prevent epinephrine inhibition of the uterus of the rabbit by ergotamine (or by ergotoxin or extract or ergot).

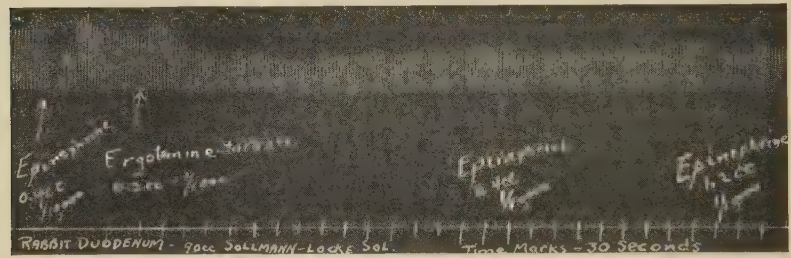


FIG. 1.
Effect of ergotamine tartrate upon the response of rabbit duodenum to epinephrine

The difference in response between the small intestine, and the colon and uterus is interesting, in view of the difference in innervation, activity and physiological stability of the organs of the upper and lower abdominal cavity.

---

[4] Mendez, R., *J. Pharm. Exp. Therap.*, 1928, xliii, 451.

* Supplied by H. A. Metz Co.

4367

Variations in the Growth-Promoting Power, for Planarian Worms, of Digestive Mucosa of Rabbit.

ALICE M. BAHRS. (Introduced by A. R. Moore.)

From the Department of Animal Biology, University of Oregon, Eugene.

Through the use of the planarian worm as the test animal, according to the method developed by R. Wulzen,[1] I have found that different regions of the digestive mucosa of the rabbit cause marked variations in growth when fed to planarian worms.

According to this method, the worms were accurately measured at the beginning and at the end of the experiment, the result being the total gain in millimeters of the experimental group. Each experimental group consisted of 30 worms, 6, 7 and 8 mm. in length, and had an average total length of 200 mm. at the beginning of an experiment. Repeated measurements have shown that the total length of an experimental group can be duplicated with no greater error than 1%. The worms were fed twice a week upon portions of the digestive tract from freshly killed rabbits and each portion was placed in the respective bowl in such a manner that the worms could feed only upon the mucous membrane. Six parts of the digestive tract were used, as follows: the posterior half of the oesophagus, the fundus of the stomach, the duodenum at the entrance of the pancreatic duct, the anterior part of the caecum and the last part of the colon having 3 bands of taenia coli. Each part was carefully washed in running tap water before being fed to the worms.

Three separate experimental series, in which different groups of worms and different rabbits were used, exhibited the same variations in growth. Fig. 1 is a graphic representation of the first series and shows the total gains in the experimental groups fed upon these 6 regions of the digestive mucosa, for a period of 3 weeks. The percentage gains in this experiment are as follows: oesophagus 15%, stomach 37%, duodenum 75%, caecum 41%, vermiform process of the caecum 101%, colon 19%. In Fig. 2, second series, there are given the growth curves of 4 groups of worms fed respectively upon the oesophagus, stomach, duodenum and vermiform process of the caecum for a period of 4 weeks. In this series the percentage gains at the close of 4 weeks are as follows: oesophagus 40%, stomach 73%, duodenum 95%, vermiform process of the caecum 118%. The same order in growth-promoting power has been maintained in both series. The third series yields the following percentage gains at the close of 3 weeks: oesophagus 12%, stomach 28%, duodenum 31%, caecum 26%, vermiform process of the caecum 53%, colon 4%. The percentage gains in this series are lower for all 6 regions of the digestive mucosa, but show the same order in growth-promoting power.

---

[1] Wulzen, R., *Science*, 1927, lxv, 331.

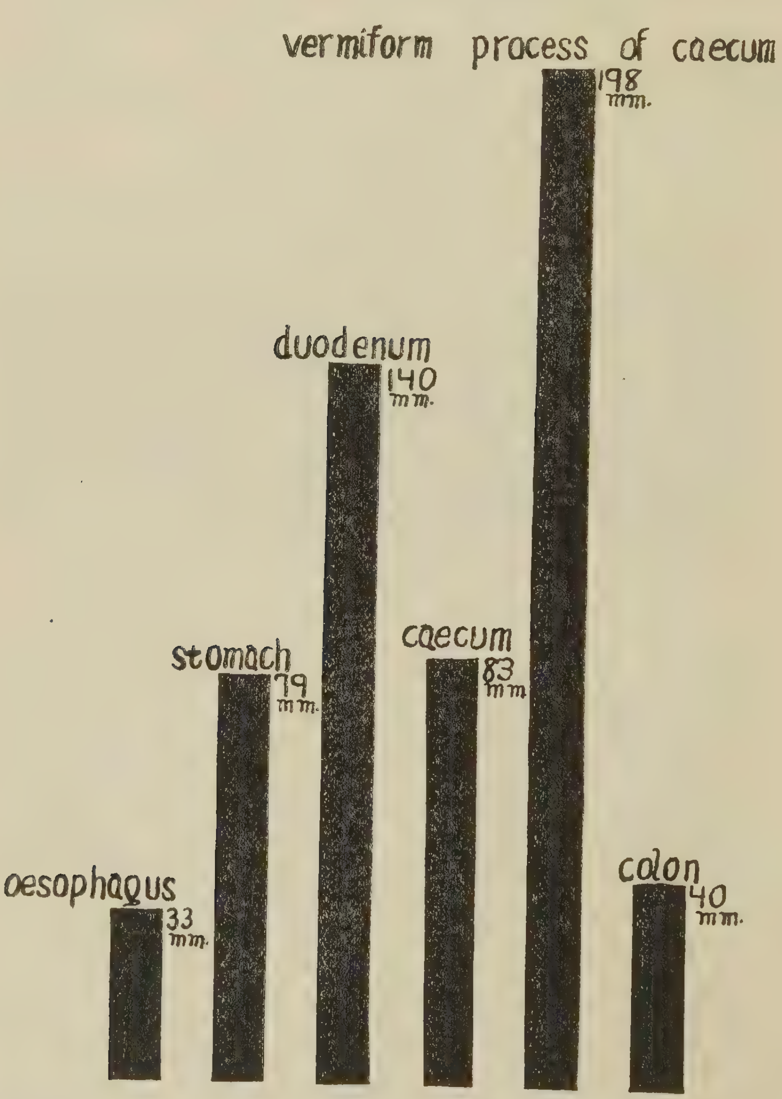


FIG. 1.

Total gains in mm. of experimental groups of planarian worms fed upon different portions of the digestive mucosa of the rabbit.

When fed to planarian worms, the mucosa from different regions of the digestive tract of the rabbit exhibits the following increasing order in growth-promoting power: oesophagus and colon < stomach and caecum < duodenum < the vermiform process of the caecum.

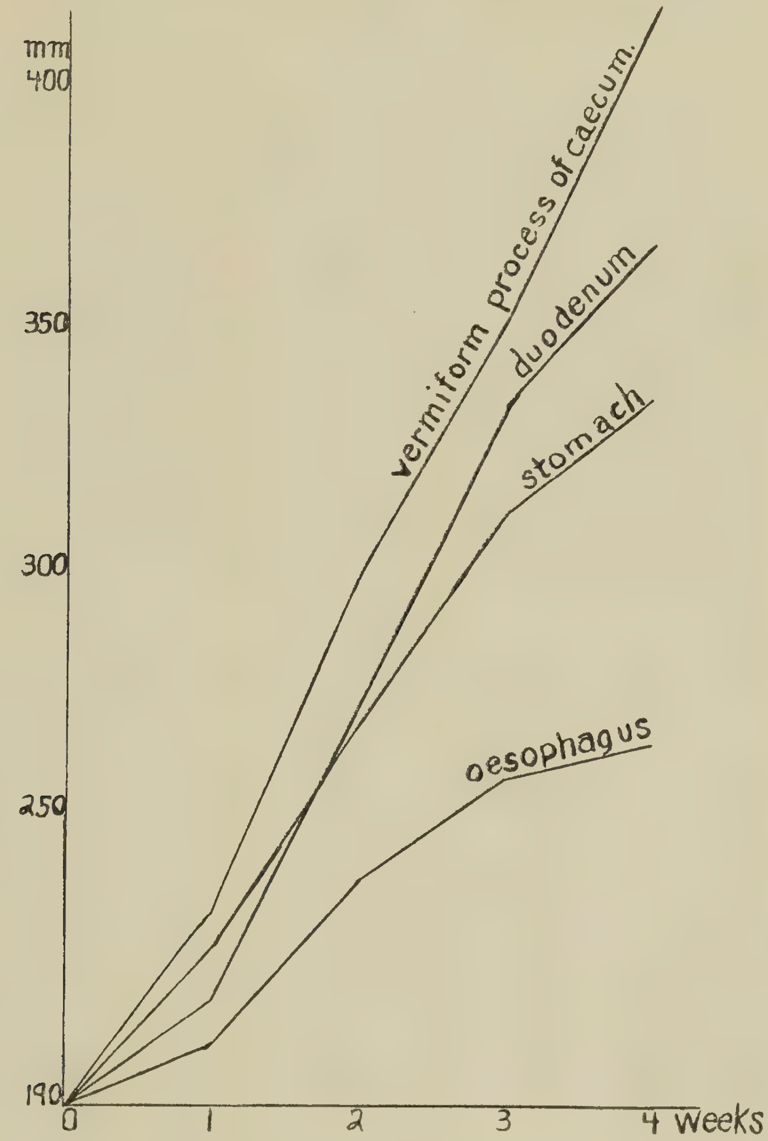


FIG. 2.

Growth curves of 4 experimental groups of planarian worms fed upon different portions of the digestive mucosa of the rabbit.

4368

Studies of the Action of Calcium on the Vertebrate Heart.

WRAY LLOYD. (Introduced by Oskar Klotz.)

From the Department of Pharmacology, University of Western Ontario, London, Ontario.

These studies are based upon a series of experiments upon the hearts of 52 animals perfused with oxygenated Locke's solution by a modified Langendorff technique. The essential features in the modification of this method were: (1) Complete water bath circulation about the perfusion fluid, maintained at a constant rate of flow by means of a pump and regulated at a constant temperature by means of a thermostat. (2) Control of the pressure head of the perfusing fluid by graduation of the pressure of flow against a mercury manometer. (3) Introduction of a warm air chamber about the beating heart. (4) The recording simultaneously of auricular and ventricular contractions by means of pins, attached through the auricles and ventricular apices. The series of animals was composed of one turtle, 2 cats, 3 dogs and 46 rabbits. Calcium was introduced into the perfusing fluid, in varying quantities of a 0.25% solution of the chloride. Observations upon the beating heart were made by direct inspection and notation at the time of experiment and by

TABLE I.

Effect.	Turtle		Cat		Dog		Rabbit	
	No. of admin.*	% occur.†	No. of admin.	% occur.	No. of admin.	% occur.	No. of admin.	% occur.
Increased strength of systole	23	73.9	27	96.3	15	86.6	453	93.7
Increased myocardial tonus	23	65.2			15	6.6	453	13.2
Bradycardia	23	13.0	27	37.0	15	33.3	453	24.7
Tachycardia	23	17.4	27	3.7	15	6.6	453	2.9
Alternating effects of Bradycardia and tachycardia	23	4.3					453	7.3
Auricular-ventricular dissociation					15	6.6	453	1.9
Fibrillation					15	13.3	453	1.3
Flutter							453	0.6
Alternation of the beat	23	4.3						
Systolic death							‡hearts 36	91.7
Diastolic death							hearts 36	8.3

* Numbers of administrations of calcium chloride.
† Percentage occurrence of effect.
‡ Number of hearts.

means of kymographic tracings. The commonest effects of the pharmacological administration of calcium upon the perfused heart are noted in the accompanying table.

As a normal amount of calcium ions is necessary for the ordinary strength of the heart's contraction so the addition of more calcium results in a greater force of both auricular and ventricular systole, often but not invariably associated with an increase of myocardial tonus. Just as Brull[1] found in the perfused hearts of dogs and rabbits, and as Bowler and Walters[2] learned by electrocardiogram studies in dogs injected intravenously with calcium chloride, large doses resulted in the production of varying types of cardiac arhthymias. Anatomically, the site of the different actions of varying amounts of calcium on the vertebrate heart has not been certain. In the excised heart it cannot be responsible to changes centrally effected in the vagus and sympathetic innervation. That these effects are probably not upon the intrinsic conducting system of the heart has been demonstrated in these experiments by section of the auriculo-ventricular bundle in the beating heart. During the course of resulting complete auricular-ventricular dissociation, the addition of calcium to the perfusing fluid has resulted, as in the intact heart, in the recorded effects of increased strength of systole, bradycardia, and with smaller amounts tachycardia in the ventricular muscle. The B.P. tincture of digitalis, diluted 1-100 with Locke's solution and mixed with the perfusing fluid in a like manner, has produced neither increased strength of systole nor bradycardia. Consequently we believe the site of action of the calcium ion to be directly upon the myocardial cell, probably upon the colloidal system of the cell membrane.

Functionally, it is manifest that all the properties of heart muscle tonicity, contractility, rhythmicity, irritability and conductivity may be effected by calcium. Of changes in rate, bradycardia has been found much the more frequent, occurring equally in both auricle and ventricle in the rabbits' heart, where the bradycardia due to a partial heart block has been a relatively infrequent occurrence. Hence we are unable to support Brull's conception in the rabbit at least, that the bradycardia following calcium administration is in most instances due to partial auricular-ventricular dissociation. We regard it rather as a myocardial effect. Andrus and Drury[3] have shown that a pH lower than normal, pH 7.0 caused the excitatory process to be propagated more slowly in the mammalian auricle. The hydrogen ion concentrations of our solutions unfortunately were not determined. Tachycardia has been observed much less often, although it has been noted on 19 occasions in the hearts of the turtle, rabbit, cat and dog. Brull regarded bradycardia as always the change in rate produced by calcium administration. The systolic death noted in the frog's heart was absent in a series of 42 experiments upon rabbits and dogs, according to the latter author's results. The state of ventricular arrest in our perfusion experiments was possible of recording upon the kymographic tracing, where the increasing tension of a rising ventricular tonus produced at the end of the experiment a vertical upstroke upon the smoked paper. This observation was verified in each instance by palpation of the ventricle and by actual measurement of its diastolic and systolic diameters with calipers. In 33 rabbits' hearts examined in this manner, the characteristic and practically constant effect has been a cessation of the heart's action in systole with a well marked calcium rigor. The failure of this effect in the perfused heart of the rabbit following treatment with calcium we believe to indicate the absence of the opportunity for the complete calcium action to have exerted itself upon the ventricular muscle.

---

[1] Brull, *Arch. internat. de méd. expér.*, 1925, 1, 613.

[2] Bowler and Walters, *J. Am. Med. Assn.*, 1924, lxxx, 111, 1232.

[3] Andrus and Drury, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 21.

4369

Anticomplementary Action of Purified Heparin.

PAUL GROSS* AND E. E. ECKER.

From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio.

The writers, in a previous paper,[1] reported on the anticomplementary action of heparin. It was found that the antihemolytic effect is not due to an action upon the erythrocytes. Although an ancillary action upon amboceptor could not be excluded, the action was found to be upon the complement. Further investigation demonstrated that the anticomplementary effect of heparin is due to its interference with the heat-stable factor or the so-called third component of complement. Complement inactivated by heat and by NH_3, containing in both instances the third component, reactivates heparin-inactivated complement. Previous admixture of a small amount of calcium to heparin destroys its anticomplementary activity without interfering with its anticoagulant action. It was found that a large amount of heparin is required to inhibit complement, many times the dose that will render a corresponding amount of blood incoagulable. This is also pointed out by Hyde.[2] Intracardiac injections of guinea pigs with as much as 50 mgm. of heparin led to incoagulability of the blood without impairment of complement titer. An anticomplementary action of heparin is reported by Fuchs and Hartman.[3] Prof. Howell's announcement[4] of the preparation of N-free heparin, suggested an investigation to determine whether or not the anticomplementary power is due to heparin *per se* or to the impurities associated with it. Furthermore, the possibility that the anticoagulant effect and the anticomplementary power depend upon 2 different mechanisms also indicated more work in that direction.

---

* Crile Research Fellow in Pathology.

[1] Ecker, E. E., and Gross, Paul, *J. Infect. Dis.*, (in press).

Through the courtesy of Prof. Howell, who kindly prepared for us a small amount of purified heparin, and to whom we are much indebted, we are enabled to report results obtained with the purified product. The anticomplementary power of purified heparin was retained and the action was again found to be upon the heat stable fraction of the complement. Complement, inactivated by heat and by NH_3, reactivates the complement inactivated by purified heparin. While 0.18 mgm. of the crude heparin inactivated one unit of complement, 0.013-0.018 mgm. of the purified product produced the same effect. The anticomplementary power of the purified heparin was increased about 10 fold. The anticoagulant power, however, sustained a 20 fold increase by the process of purification. Prof. Howell, in this instance, obtained 0.060 gm. N-free heparin from 3 gm. commercial heparin. Weight for weight, the purified product is from 10 to 20 times more active than the original preparation.

Conclusion: Heparin, purified by the recent method of Howell, operates upon complement in the same qualitative manner as does unpurified heparin, namely upon the third component. The amount of purified heparin necessary for effect is much reduced as compared with the unpurified product. The purified product is definitely increased in activity.

---

[2] Hyde, R. R., *Am. J. Hygiene*, 1928, viii, 859.

[3] Fuchs, H. J., and Hartman, E., *Ztschr. f. Imm.*, 1928, lviii, 1.

[4] Howell, W. H., *Bull. Johns Hopkins Hosp.*, 1928, xlii, 199.

4370

Blood Regeneration in Nutritional Anemia. Influence of Iron, Iron and Copper, Nickel, Cobalt, Germanium, or Sodium Germanate.

HOWARD H. BEARD AND VICTOR C. MYERS, with the assistance of Reginald A. Shipley.

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

Young rats were made anemic by feeding whole milk for a period of 6 weeks after weaning. When the erythrocyte count was about 3-4 million per cu. mm., and the Hb content 4-6 gm. per 100 cc., additions of Fe, Fe + Cu, Fe + Ni, Fe + Co, and Fe + Ge, Fe + Na_2GeO_3 were made. Erythrocyte counts and Hb estimations were made weekly on a few drops of blood obtained by clipping the end of the tail. A Levy counting chamber with double Neubauer ruling was used. Hb determinations were made by the acid hematin method, color comparison being made with a calibrated Newcomber disc. The results are summarized in the table.

When 0.5 mg. Fe was given daily to anemic rats recovery of both erythrocytes and Hb was complete in 4.3 and 6 weeks; with 1 mg. the time for recovery was 3.5 and 4.8 weeks; and with 1.5 mg. it was 3.5 and 3.7 weeks, respectively. Since this iron solution was prepared from standard iron wire using hydrochloric and nitric acids, it would hardly seem that the effect of iron salts, as $FeCl_3$, in blood regeneration could be due to the impurities these salts might contain, as Hart and coworkers suggest.[1] Mitchell and coworkers[2] had previously shown that some iron salts, *e. g.*, $FeCl_3$, were effective in blood regeneration in rats fed whole milk diets. Our results seem to confirm those of Mitchell in respect to this one iron salt. The experiments also appear to confirm those of Hart and his coworkers[3] on the supplementing action of copper when added to iron, but do not indicate that copper is unique in this respect.

The data in the table show that traces of copper, nickel, cobalt, germanium and sodium germanate plus 0.5 mg. Fe all brought about regeneration of erythrocytes and hemoglobin in less time than did 0.5 mg. iron alone. A number of other elements have been studied.

---

[1] Hart, E. B., Elvehjem, C. A., Waddell, J., and Herrin, R. C., *J. Biol. Chem.*, 1927, lxxii, 299.

[2] Mitchell, H. S., and Schmidt, L., *J. Biol. Chem.*, 1926, lxx, 471; Mitchell, H. S., and Vaughn, M., *J. Biol. Chem.*, 1927, lxxv, 123.

[3] Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, lxxvii, 797.

TABLE I.
Average Values.

No. of Animals	Body Wt.		Erythrocytes		Hemoglobin		Time for Recovery		Daily Diet of Whole milk plus
	Beginning	End	Beginning	End	Beginning	End	Erythrocytes	Hemoglobin	
	gm.	gm.	million cu. mm.		gm. 100 cc.		weeks	weeks	
11	97.0	139.0	4.1	8.5	5.2	14.0	4.3	6.0	0.5 mg. Fe
6	105.0	144.5	4.5	8.3	5.3	14.2	3.5	4.8	1.0 mg. Fe
3	95.0	121.5	3.4	8.5	4.1	13.7	3.7	3.7	1.5 mg. Fe
10	88.0	112.5	4.4	8.4	5.7	14.2	2.5	2.9	0.5 mg. Fe + 0.05 mg. Cu
2	57.0	100.0	4.0	9.9	6.9	14.3	1.5	3.0	0.5 mg. Fe + 0.05 mg. Ni
2	61.0	94.0	3.2	9.0	5.5	13.6	2.0	3.0	0.5 mg. Fe + 0.1 mg. Ni
2	48.0	86.0	3.0	8.2	5.0	14.0	3.0	5.0	0.5 mg. Fe + 0.05 mg. Co
2	53.0	72.0	4.0	8.7	6.3	11.1*	3.0	4.0	0.5 mg. Fe + 0.1 mg. Co
7	104.0	126.5	4.8	8.4	6.1	13.7	2.4	2.6	0.5 mg. Fe + 0.5 mg. Ge
4	75.0	137.0	2.9	8.6	4.7	13.4	2.8	4.8	0.5 mg. Fe + 0.05 to 0.2 mg. Na_2GeO_3

* Experiment still in progress.

4371

Effect of Calcium on the Action of Pilocarpine and Atropine.

WILLIAM SALANT AND HENRY WASHEIM, JR.

From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Ga.

When segments of the small intestine of the cat or rabbit are suspended in calcium free Tyrode solution, rhythmic contractions as well as tonus are abolished. The intestinal movements may be stimulated, however, by pilocarpine. A concentration of 1-500,000 was sufficient to provoke rhythmic contractions of moderate force which became much stronger with increasing amounts of pilocarpine. In no case, however, did the amplitude attain the size produced by the same concentrations in normal Tyrode solution. The rate was also slower, the latent period was considerably longer, usually about one minute, while tonus, with all concentrations, was very low. The movements were markedly stimulated, the tonus being enormously increased when a small quantity of calcium chloride was added to the calcium free solution containing pilocarpine in a concentration of 1-500,000. But the same amount of calcium produced only a very slight increase of tonus and provoked slight rhythmic contractions in the intestine suspended in calcium free Tyrode without pilocarpine. The combined action of calcium and pilocarpine is, therefore, not one of summation but is apparently due to potentiation. Excessive amounts of calcium likewise decrease intestinal motility. The addition of calcium making a concentration of 1-2,000 abolished the contractions produced by pilocarpine in Tyrode minus calcium.

Experiments were also made on the effect of calcium on the action of pilocarpine on the isolated frog heart. Calcium deficiency produced the usual effect. In some cases, however, heart action, though feeble, continued for a few minutes. The presence of pilocarpine in Ringer solution containing no calcium or minimal amounts only was without effect. But in several of these experiments in which atropine in concentration of 1-500,000 was perfused heart action was distinctly slowed. This reversal of effect occurred when pilocarpine was perfused before atropine as well as when no pilocarpine was used. In experiments in which the amount of calcium in Ringer solution was increased 3 times the following results were obtained. A concentration of 1-100,000 pilocarpine in normal Ringer reduced the force of the heart beat 50-60% in 7-12 minutes and decreased its frequency nearly 14%. The same amount of pilocarpine in Ringer containing an excess of calcium produced only a moderate decrease of the amplitude in some experiments and scarcely any change in others, while the rate was decreased 21.5%.

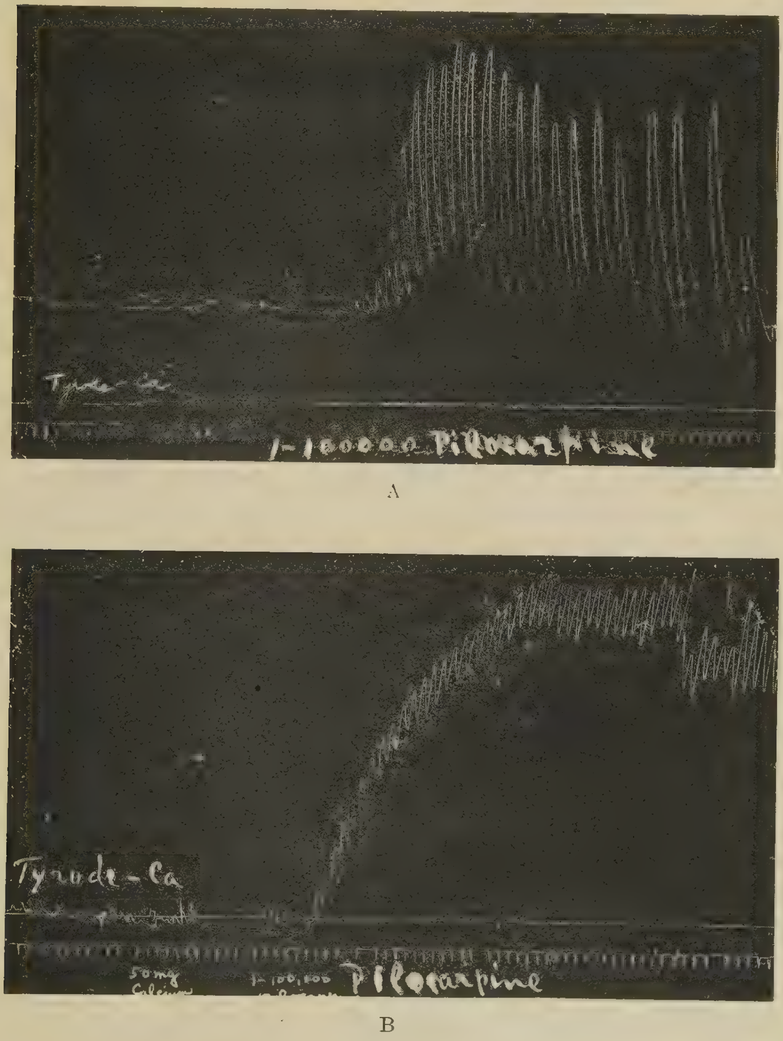


FIG. 1.

A. Expt. 18. Intestinal movements disappeared when segments of the cat's intestine were suspended in Locke's solution minus calcium. The addition of pilocarpine to the solution stimulated the rhythmic contractions but not the tonus.
B. Expt. 26. The same concentration of pilocarpine as in A stimulated the tonus after calcium was added to the solution.
Reduced one third.

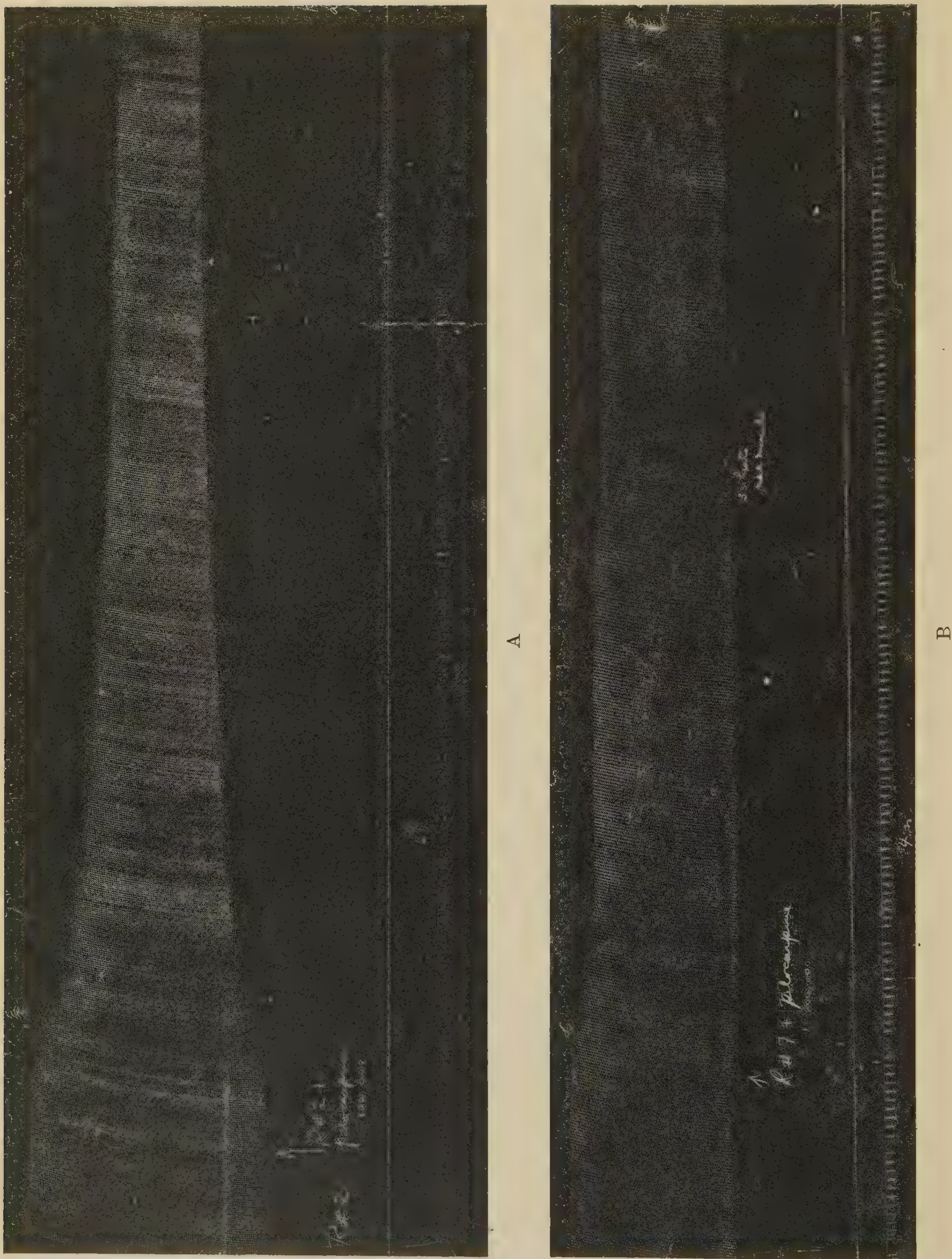


FIG. 2.

A. Expt. 9. Frog's heart perfused with normal Ringer's solution containing 1-100,000 pilocarpine. B. Expt. 15. Shows that the same concentration of pilocarpine in Ringer's solution containing three times the amount of calcium as in A produced only a slight decrease in the strength of the contractions, but the slowing was greater than in A.

Reduced one third.

Summary: 1. Pilocarpine stimulates intestinal movements in the absence of calcium in the surrounding medium, but tonus is only slightly increased. 2. Excess calcium depresses the effect of pilocarpine on the intestine. 3. Pilocarpine sensitizes the intestine to calcium. 4. Pilocarpine in Ringer minus calcium is without effect on the isolated frog heart. 5. Excess calcium antagonizes the depression produced by pilocarpine. 6. The action of atropine is reversed by calcium deficiency, thus causing a slowing of the heart.

4372

The Iodine Values of "Physiological" or "Synthetic" Rat Fat.

AVA JOSEPHINE MCAMIS,* WILLIAM E. ANDERSON, AND
LAFAYETTE B. MENDEL.

From the Laboratory of Physiological Chemistry, Yale University, New Haven.

Male albino rats taken at weaning when 21 days of age were fasted for 2 days in order that a partial depletion of the stored fat might ensue, thereby lessening its influence on the later resulting "synthetic" fat. The rats were then fed a diet consisting of 18% casein (extracted with alcohol and ether), 77% sucrose, and 5% salt mixture (Osborne and Mendel), supplemented daily by the following: a hot water extract of pig's liver (≈ 0.4 gm. dried liver), 100 mgm. of yeast concentrate (Harris), 20 mgm. of a non-saponifiable fraction of cod liver oil (a pasty material, Oscodal†—Iodine Number 110), and 0.016 mgm. of irradiated ergosterol (dissolved in alcohol). Four other groups of rats (5 or more in a group) were fed this "fat free" diet modified so that the first group received an addition of 20 mgm. of peanut oil (Iodine Number 84); the second had the non-saponifiable fraction of cod liver oil substituted by 20 mgm. of cod liver oil (Iodine Number 160); the third received the same diet as the second group with the addition of 20 mgm. of peanut oil; and the last had the extracted casein replaced by commercial casein, the yeast concentrate substituted by yeast and the irradiated ergosterol omitted.‡ From the analyses of the dietary components, it is calculated that the rats on the "fat free" diet received daily probably less than 7 mgm. of fatty materials, other than the non-saponifiable matter from cod liver oil. At stated intervals, the rats were killed and the fat from the entire animal, with the exception of the alimentary tract, was rendered out by essentially the technic used by Anderson and Mendel,[1] modified slightly in order to obtain a more complete extraction of the fat. The fat yielded by the rats grown on a diet practically devoid of "true" fats, which might be designated as "physiological" or "synthetic" rat fat gave iodine values of 64 to 71 by the Hanus method. There was little difference in the iodine numbers of the fats obtained from the rats grown on the "fat free" diet and from those animals whose diets contained a small inclusion of fat.

---

* Holder of the Alpha Xi Delta Fellowship awarded by the American Association of University Women (1928-29).

† Kindly supplied by Dr. H. E. Dubin of the H. A. Metz Laboratories.

‡ A paper is now in press in the *J. Biol. Chem.* on the "Growth of Rats on 'Fat Free' Diets."

These experiments indicate that the fat produced *de novo* in the body out of non-fat precursors has presumably a definite characteristic chemical make-up, which is not influenced materially by the inclusion of a very small amount of fat in the diet, as judged by the iodine numbers of the total body fat. An examination of the samples of fat obtained from the rats grown on the diets mentioned, for their constituent fatty acids, and the extraction of the fatty materials from the feces, is now in progress.§

4373

Studies on the Pathogenesis of Endocarditis.

KURT SEMSROTH AND ROBERT KOCH. (Introduced by Ralph Mellon.)

From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh, Penn.

In view of the well known multiplicity of bacteria isolated from endocarditic lesions, workers in this field have always more or less distinctly felt that the essential question in the pathogenesis of endocarditis is one of bacterial *localization.* Under what conditions will bacteria localize on the endocardium? While early experimental investigators (Wyssokowitsch,[1] Ribbert[2]) succeeded in producing an endocarditis by combining the bacterial infection with a gross trauma to the heart-valves, observations on experimental endocarditis without such trauma (Lissauer,[3] Saltykow[4]) have been rare until recently. Birkhaug[5] produced a subcutaneous lesion with a streptococcus infected agar focus, and obtained endocarditis with regularity by intravenous reinfections with the same organism. He interprets these results in the sense of an etiological specificity of the organism employed. Siegmund[6] produced experimental endocarditis by daily intravenous injections of rapidly increasing doses of *B. coli* or staphylococcus. Siegmund[6] and Dietrich[7] conclude that a peculiar state of immunity, associated with an altered capacity of the endocardium to react, is the essential condition for bacterial localization on the endocardium.

---

[1] Anderson and Mendel, *J. Biol. Chem.*, 1928, lxxvi, 729.

§ Since this report was sent to press, a paper by H. C. Eckstein, dealing with synthetic rat fat has appeared in the *J. Biol. Chem.*, lxxi, 613. The iodine values reported in the 2 papers are in general agreement.

In the course of observations on infectious disease processes in horse serum sensitized guinea pigs we found, that on single intraperitoneal injection of salmonella or *Staphylococcus aureus*, sensitized guinea pigs developed with considerable regularity an endocardial lesion, which was only occasionally met with in non-sensitized controls. The lesion consists of nodular monocytic proliferations—"proliferative endocarditis". Desensitization previous to the infection practically prevented the above nodular reaction of the endocard, desensitization after the infection was without detectable influence.

As a sequel of horse serum sensitization the capacity of the endocardium to react against an infection had evidently been altered. In an attempt to gain information about the possible significance of these results the above morphological findings were compared with earliest endocardial reactions in human endocarditis as well as with such reactions in an experimental rabbit's endocarditis. (Streptococcus vaccination, subsequent staphylococcus infection, after Freifeld[8]).

It was found that in the experimental rabbit's endocarditis as well as in human endocarditis nodular monocytic proliferations represent earliest endocardial reactions.

The close morphological analogy between the endocardial reaction of the horse serum sensitized guinea pig and early endocardial reaction in endocarditis suggested that processes somehow related to horse serum sensitization, are an essential condition for bacterial localization on the endocardium.

---

[1] Wyssokowitsch; [2] Ribbert; [3] Lissauer; [4] Saltykow. Reviewed in *Henke-Lubarsch Handb. d. spez. path. Anat.*, II, 205-206.

[5] Birkhaug, *J. Inf. Dis.*, 1927, xl, 549.

[6] Siegmund, *Verhdl. d. deutsch. path. Gesellschaft*, 1923, xix, 114.

[7] Dietrich, *Verhdl. d. deutsch. path. Gesellschaft*, 1925, xxxvii, 188.

[8] Freifeld, *Klin. Wochenschrift*, 1928, vii, 1645.

In an effort to throw more light on the question under consideration, an allergic state was induced in rabbits by 5 or 10 successive daily intravenous injections of 2 cc. of 2% casein in 0.1% of sodium carbonate. It was found that after 5 to 10 casein injections 10 out of 15 animals showed a definite, 5 a less definite, *hyperergic skin reaction* to injection of casein. No morphologically detectable effects of the casein treatment, especially on the endocardium, were found in controls with the usual histological methods. On intravenous injection of 1 to 2 cc. of an 18-hour *Staphylococcus aureus* culture 5 out of 7 animals developed a definite endocarditis.

We feel justified to draw the inference that bacterial localization on the endocardium—in our experience—is due to an allergic state of the endocardium, *i. e.*, an altered capacity to react.

It is also to be inferred that a relationship between that allergic state and the subsequent infection in the sense of species-specificity is not a requirement for bacterial localization on the endocardium, and that furthermore, that allergic state may be induced by a non-bacterial foreign protein-matter. The immun-biological definition of that allergic state remains a matter of further study.

4374

Adaptation of the Bloor Oxidation Procedure to the Determination of Small Quantities of Cholesterol as Digitonid.

RUTH OKEY.* (Introduced by W. R. Bloor.)

From the Department of Biochemistry, School of Medicine and Dentistry, University of Rochester.

Micro methods for the estimation of cholesterol have hitherto been based almost entirely upon the color reaction with acetic anhydrid and concentrated sulphuric acid. The presence of interfering color producing substances in blood and tissue extracts frequently makes it highly problematical whether or not this reaction gives a true picture of the cholesterol concentration. The gravimetric estimation as the digitonid, proposed by Windaus[1] is impracticable where the amounts to be weighed are as small as those available in the average blood analysis.

We have, therefore, attempted to adapt the digitonin precipitation to micro determination by oxidation of the digitonid according to the method used by Bloor[2] for fatty acids and phospholipins; *i. e.*, with silver-chromate sulfuric acid (Nicloux reagent) and normal $K_2Cr_2O_7$. Since this oxidation is not specific, the largest problem involved has been the separation of the digitonid from the excess digitonin used in precipitation, and from contaminating substances of a fatty nature. Digitonin is a saponin. Consequently, the surface tension of its water solutions is such as to make separation of the digitonid by centrifugalization impossible. Filtration of the digitonid from water solutions of digitonin has proved quantitative. However, the physical nature of the digitonid precipitate is such as to make quantitative removal of very small amounts from the filter and sides of the flask impracticable. We have, therefore, devised, with the assistance of Mr. D. J. Kooyman, a small Gooch filter tube made from a piece of Pyrex glass tubing 45 mm. long and 18 mm. in diameter, constricted somewhat at one end and fitted with a perforated porcelain disk (cut from the bottom of an ordinary Gooch crucible). This, after the filtration and washing is completed, may be placed bodily inside the flask used for the oxidation in such a way that the oxidizing solution may be made to come in contact with every part of the tube and the asbestos mat. The asbestos used for the filter must be prepared by long continued (72 to 120 hrs.) heating at 120-130° C. with bichromate-sulphuric acid which is frequently renewed. The asbestos is washed free of bichromate and suspended in distilled water in the usual way. This filter differs from that used by A. von Szent Györgyi[3] in that it makes possible complete oxidation of the digitonid by allowing control of time and temperature.

---

* On leave, University of California, Department of Household Science.

[1] Windaus, *Z. f. Physiol. Chem.*, 1910, lxv, 110.

The procedure consists, briefly, of (a) precipitation of the digitonid by adding alcoholic digitonin solution to a measured amount of the cholesterol containing extract in the oxidation flask, (b) evaporation just to dryness, (c) removal of fatty substances by washing with anhydrous alcohol-free ethyl ether, pouring off the ether through the prepared filter tube described above, (d) removal of excess digitonin and water soluble impurities by washing repeatedly with hot and cold distilled water, filtering through the same tube into a fresh flask (to avoid back pressure of ether vapor), (e) transfer of the tube back to the original stoppered Pyrex Erlenmeyer, washing the mouth of the flask and the stirring rod with 1 cc. of concentrated H_2SO_4, (f) addition of the measured Nicloux reagent and normal $K_2Cr_2O_7$, as described by Bloor. A control containing a filter tube, asbestos mat, the sulfuric acid and the oxidizing agents is prepared at this time, (g) heating to 90° C. for 1½ hours to 120° to 125° C. for 15 minutes, (h) dilution, addition of KI, and titration of the excess $K_2Cr_2O_7$ with thiosulfate.

---

[2] Bloor, W. R., *J. Biol. Chem.*, 1928, lxxvii, 53.

[3] Györgyi, A. von Szent, *Biochem. Z.*, 1923, cxxxvi, 1123.

The accuracy to be expected from the method may be judged from the following table. Theoretically, if the digitonid has the formula $C_{82}H_{140}O_{29}$, 1 mg. of cholesterol should require 10.62 cc. of N/10 $K_2Cr_2O_7$ for oxidation. If, however, the cholesterol is crystallized from alcohol and contains alcohol of crystallization, the value is reduced to 9.51 cc. N/10 $K_2Cr_2O_7$.

TABLE I.

Date	Cholesterol* taken	Cc. N/10 $K_2Cr_2O_7$ used per mg.	Cholesterol Found		Deviation from theory	Variation from average	
Feb.			mg.	%	%	%	
12	1 mg.	10.30	0.969	96.9	—3.1	—0.3	water bath
		10.10	0.951	95.1	—4.9	+2.5	1 hour
13	1 mg.	10.36	0.976	97.6	—2.4	—1.0	
		10.32	0.972	97.2	—2.8	—0.6	
		10.22	0.962	96.2	—3.8	+0.4	
		10.32	0.972	97.2	—2.8	—0.6	
		10.27	0.967	96.7	—3.3	—0.1	
14	1 mg.	10.57	0.958	95.8	—4.2	+0.8	
		10.22	0.962	96.2	—3.8	+0.4	
15	1 mg.	10.22	0.962	96.2	—3.8	+0.4	
		10.27	0.967	96.7	—3.3	—0.1	1 hour
		10.13	0.954	95.4	—4.6	+1.2	20 min.
		10.04	0.946	94.6	—5.4	+2.0	
18	0.5 mg.	10.38	0.484	96.8	—3.2	—0.2	
		10.38	0.484	96.8	—3.2	—0.2	1 hour
		10.56	0.497	99.4	—0.6	—2.8	20 min.
		10.38	0.484	96.8	—3.2	—0.2	
				Averages	—3.4	0.76%	

* Cholesterol crystallized from petroleum ether.

It is our purpose to adapt the method to estimation of free cholesterol, "total cholesterol" and total unsaponifiable matter in blood and tissue.

4375

Molasses, Sorghum, and Honey as Sources of Vitamin E.

M. W. TAYLOR and V. E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College.

Previous work in this laboratory has shown that cane molasses is a good source of vitamin B. Data accumulated during the past year show that it is also a good source of the reproductive vitamin. The following products have been studied for their content of vitamin E: cane molasses or black strap, 3 kinds of beet molasses, sorghum, and honey. The 3 different beet molasses studied were: straight beet, Steffen, and barium residue molasses.

Crude cane or black strap molasses when added to a synthetic diet to the extent of 3% furnishes a good source of vitamin E. Five per cent in the ration proved ample not only for reproduction but also for rearing of young. Ninety-two per cent of the young were weaned on the 5% level and 58% on the 3% level. Beet molasses and sorghum contain some vitamin E but much less than cane molasses. The straight beet molasses was richer in vitamin E than the other kinds of beet molasses. No young were obtained on rations containing honey equivalent to 3 and 5% of the ration. The basal diet employed in these experiments consisted of purified casein 18, salt mixture (185) 3.7, filtered butter fat 5, yeast 12, and dextrin to 100. First litters are invariably born on this diet and since no young were born on the diets containing honey it may be that this is due to an anti-vitamin E factor.

The experiments in this paper employed 12 animals on the control and 12 animals on each of the different products tested. Since each product was run at two levels (5 and 3%) six animals were employed on the experimental rations on each level.

4376

Effect of Different Levels of Protein Intake on Reproduction.

RUTH FOUST and V. E. NELSON.

From the Laboratory of Physiological Chemistry, Iowa State College, Ames, Iowa.

Considerable work has been done on the effect of high protein diets on growth and general well being. The effect of high protein diets on the kidneys has also been studied. The data of Drummond,[1] Osborne and Mendel,[2] and McCollum[3] and their associates have largely been confined to these phases of study. Comparatively little has been done on reproduction.

The diets employed by us consisted of casein from 18 to 79.3%, salt mixture (185) 3.7%, wheat embryo 12%, cod liver oil (Squibb) daily 5%, and the remainder of the ration was composed of dextrin to 100%. The following levels of casein were studied: 18, 25, 30, 40, 50, 60, 75.3 and 79.3%. Growth was normal on levels of 18 to and including 60% casein but below normal on higher levels. Reproduction was good on all levels of casein studied, but was delayed on the levels above 60%. No young were weaned on the 79.3% level. Young were weaned on the lower levels and most successfully on the 18% level of casein. The young weaned on the 18 and 25% levels were normal in weight but on higher levels of casein growth of the young was stunted.

The experiments employed 7 animals on each lot, 3 males and 4 females. In other words there were 7 animals on each level of protein.

4377

Effect of Toxins and Venoms upon Protozoa.

CHARLES H. PHILPOTT. (Introduced by J. J. Bronfenbrenner.)

From the Department of Bacteriology and Immunology of the Washington University Medical School and the Zoological Laboratory of the University of Missouri.

Since the completion of a previous study on the effect of pathogenic bacteria and bacterial toxins upon paramecia,[1] an investigation has been in progress on the effect of snake venoms and of toxins (tetanus, ricin and botulinus) upon protozoa. The recent appearance of Tunnicliff's related study with other toxins,[2] and particularly her consideration of the use of paramecia for determining the strength of antitoxins, has made advisable the recording of a preliminary report of the present investigation in so far as it is related to her findings.

---

[1] Drummond, J. C., Crowden, G. P., and Hill, E. L. G., *J. Physiol.*, 1922, lxvi, 413. Reader, V., and Drummond, J. C., *Biochem. J.*, 1926, xx, 1256.

[2] Osborne, T. B., Mendel, L. B., Park, E. A., and Winternitz, M. C., *J. Biol. Chem.*, 1926-27, lxxi, 317.

[3] Polvogt, L. M., McCollum, E. V., and Simmonds, Nina, *Johns Hopkins Bull.*, 1923, xxxiv, 168.

[1] Philpott, C. H., *J. Morph. and Physiol.*, 1928, xlvi, 85.

[2] Tunnicliff, Ruth, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 213.

In the study of the effect of tetanus and botulinus toxins, of ricin and of snake venoms upon protozoa, only the venoms have definitely given positive results. *Crotalus atrox* (Texas rattlesnake) venom,* in dilutions containing 0.00025 gm. per cc, had an immediate lethal effect upon *Paramecium caudatum, Stentor coeruleus, Bursaria truncatella* and *Frontonia leucas*. With *Volvox spermatosphara* and *Oxytrichia fallax* the venom was lethal in effect but very slow in action. The effect on *Chilomonas paramecium* was slight and only temporary while *Coleps hirtus, Podophyra fixa,* and *Dileptus gigas* were not affected.

A race of *Paramecium caudatum* has thus far lent itself well to and has given consistent results in measurements of the potency of snake venoms. The M.L.D. of *Crotalus atrox* venom for the race of *Paramecium caudatum,* in this case the least amount in one cubic centimeter of medium required to kill an animal in 24 hours, was 0.00002 gm. This was one twentieth of the least amount required to kill a 20 gm. mouse. The M.L.D. of *Agkistrodon piscivorous* (Mocassin) venom for *P. caudatum* was 0.0000014 gm., while for *Bothrops atrox* (Fer de lance of Central America) it was 0.0000125 gm. It is evident that the agent in these venoms that is principally responsible for the death of paramecia is thermolabile. It is hoped that further fractionation may make possible the study of the effect of each of the several constituents of venom upon protozoa.

Antivenins neutralize the constituent in *Crotalus* venom that is lethal to paramecia. This makes possible the use of paramecia in the titration of antivenins. Titrations have been made and repeated with consistent results. Further study is being made to determine the reliability of paramecia in these titrations and to compare titers obtained with paramecia and with mice.

A certain degree of resistance to *Crotalus atrox* venom has been developed in races of *P. caudatum* by growing the animals from day to day in media containing increasing amounts of venom. The resistance developed in 17 days enabled animals to grow in the presence of 3 lethal doses of the venom. This resistance is specific for *Crotalus atrox* venom and does not protect from Mocassin or Bothrops venom. The resistance disappeared after the race grew for 13 days in venom-free media. Study is in progress to determine more definitely the mechanism of this resistance.

---

* The venoms, in desiccated form and the antivenins used in this study were supplied through the courtesy of the Antivenin Institute of America at Glenolden, Pa.

Pacific Coast Branch.

University of California School of Medicine, February 27, 1929.

4378

The Circulatory Changes Following Full Therapeutic Doses of Digitalis.

W. DOCK AND M. L. TAINTER.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, San Francisco, California.

It has been thoroughly established that digitalis diminishes cardiac output of normal animals. Recent observers have attributed this to a direct cardiac action of digitalis,[1, 2] but earlier workers[3, 4] showed that the action on the peripheral vessels was of greater importance. The relationship of the vascular changes to cardiac output in intact animals has not been clearly established or appreciated. We have measured the cardiac output of dogs by the Fick and cardiometric methods and have also observed the changes in arterial pressures, and peripheral organ volumes.

Full therapeutic doses of digitalis produce the following changes in the circulation of morphinized dogs: There is an immediate rise of arterial and venous pressures together with a vagal slowing of the heart, and a peripheral vasoconstriction. Within 5 minutes the venous pressure begins to fall, although arterial pressure may rise gradually for over half an hour. Severe operative procedures diminish the pressor responses. The diminution in cardiac output continues with the progressive fall in venous pressure for 2 hours or more. The vessels of the skin and intestine remain constricted, but the spleen and liver increase in volume. The latter change indicates a pooling of blood in this reservoir probably as the result of hepatic vein constriction which thus results in diminished venous return to the heart.

---

[1] Harrison, T. R., and Leonard, B. W., *J. Clin. Invest.*, 1926, iii, 1.

[2] Cohn, A. E., and Stewart, H. J., *J. Clin. Invest.*, 1928, vi, 53.

[3] Kaufmann, M., *Rev. d. Med.*, 1884, iv, 381.

[4] Tigerstedt, K., *Skand. Arch. Physiol.*, 1908, xx, 115.

It is therefore concluded that the diminution of cardiac output following digitalization of dogs is due to diminished filling of the heart, as the result of diminished venous return, which in turn is due to widespread peripheral vasoconstriction and redistribution of blood. There is no evidence that the reduced blood flow is due to a cardiac action of the drug.

4379

Relation of Protein Denaturization Rate to Specific Antibodies.

W. H. MANWARING, J. L. AZEVEDO AND T. H. BOONE.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Horse proteins injected in routine doses intravenously into normal dogs are so completely denatured by the end of about 4 days that they no longer call forth recognizable anaphylactic reactions on massive blood transfusion into partially exsanguinated horse-serum-hypersensitive dogs.[1] Representing this normal denaturization rate by the coefficient 1.0, the following approximate coefficients summarize our present data:

Horse protein denaturization rate in:

(a) Normal dogs	1.0
(b) Horse-serum-immune dogs[2]	0.7
(c) Cow-serum-hypersensitive dogs	0.5
(d) Horse-serum-hypersensitive dogs[1]	6.0 to 8.0
(e) Desensitized H.S. hypersensitive dogs	4.0

The rapid protein denaturization in hypersensitive dogs, therefore, is apparently due to some specific antibody which is absent or inoperative in immune dogs. This is best explained by the assumption that anaphylactic antibodies and immune antibodies are of different chemical compositions and of different physiological functions, both conceivably defensive in character.

---

[1] Manwaring, W. H., *et al.*, *J. Immunol.*, 1927, xiii, 357.

[2] Manwaring, W. H., *et al.*, *J. Immunol.*, 1928, xv, 351.

4380

Toxicity of "Eucupin" in Local Analgesia.

RAYMUND J. MILLZNER AND CHAUNCEY D. LEAKE.

From the Departments of Surgery and Pharmacology, University of California Medical School.

A group study for the evaluation of local analgesics by clinical trial was organized at the University of California Hospital in December, 1927. Prolongation of analgesia was especially desired. The analgesics first studied were 0.5% and 1.0% procaine and "tutocaine" to which 0.1% "eucupin" had been added, as suggested by De Takats.[1] These solutions gave extremely satisfactory analgesia, lasting in some cases as long as 24 to 36 hours. Local reactions, such as erythema, induration, and delayed healing occurred, however, in the majority of cases. Extensive sloughs of the skin and subcutaneous structures that greatly prolonged the post-operative course, developed in 2 out of 15 patients. Four others suffered minor sloughing.

These reactions suggested excessive toxicity for the infiltrated structures and made the abandonment of the "eucupin" containing solutions advisable. As a further check, a series of controlled intradermal wheals were made, using 10 medical students as subjects. The solutions used were 1% procaine and 1% "tutocaine" to which 0.1% "eucupin" had been added, and solutions of 1% procaine and 1% "tutocaine" for controls. The results checked the clinical observations. The average duration of analgesia for the 1% procaine and the 1% "tutocaine" was 2 hours. The average duration of analgesia for the 1% procaine-0.1% "eucupin" mixture was 20 hours; for the 1% "tutocaine"-0.1% "eucupin," 24 hours. There was no evidence of local tissue damage at the site of either the procaine or "tutocaine" injections. Local reactions occurred in 6 cases (60%) with each of the "eucupin" solutions. These varied in intensity from a persistent erythema to definite sloughing. There were 2 small sloughs from the procaine-"eucupin" and 3 sloughs from the "tutocaine"-"eucupin". Of the latter, 2 were quite small, but the third was nearly 2 cm. in diameter and accompanied by marked vesication and erythema. All were non-painful. Pigmentation and partial analgesia were still present 8 months later at the sites of 4 of the procaine-"eucupin" and 5 of the "tutocaine"-"eucupin" wheals.

---

[1] Takats, G. De, *Surg., Gynec. and Obst.*, 1926, xliii, 100.

Further tests were carried out using 0.05% and 0.01% "eucupin" with both procaine and "tutocaine". These weaker solutions gave no definite prolongation of analgesia beyond that of the controls and gave no local reactions.

All of the solutions were prepared by the Pharmacy of the University of California Hospital. "Eucupin base" was used throughout. It was dissolved by the addition of 0.1 N HCl and the solution was then neutralized with 0.1 N NaOH until the mixture remained persistently cloudy. The addition of 2 or 3 drops of 0.1 N HCl then sufficed to clear the solution by redissolving the precipitated "eucupin base".

Our conclusion is that, although solutions containing "eucupin" give satisfactorily prolonged analgesia, the local toxicity is too great for clinical use. Solutions made directly from "eucupin dihydrochloride" might possibly prove less toxic and tests are now in progress to determine this.

4381

X-Ray Diffraction Spectra of Glycine.

MAURICE H. SIMMERS. (Introduced by S. C. Brooks.)

From the Department of Zoology, University of California.

There still remains some uncertainty[1] as to whether the needle-like crystals of glycine (produced by precipitation with alcohol from aqueous solution) are identical with the plate form of the crystals (produced by crystallization from water).

This investigation attempts to throw some light on the problem by making use of X-ray diffraction patterns; the powder method was employed.

Glycine from 4 different sources, as follows, was used. A—Prepared by Pfanstiehl. B—Prepared by Eastman Kodak Company. C—Prepared at the Biochemistry Department of this University by the formaldehyde method.[2] D—"Dunn No. 1, 1927," prepared by the reaction of monochloracetic acid and ammonia. The samples, after thorough drying, were divided into 3 groups and diffraction patterns taken as follows:

---

[1] Falk and Sugiura, Proc. Soc. Exp. Biol. and Med., 1917, xv, 25. Falk and Sugiura, *J. Biol. Chem.*, 1918, xxxiv, 29. Biltz and Paetzold, *Ber. deutsch. chem. Ges.*, 1922, xvi, 1066. Brautlecht and Eberman, *J. Am. Chem. Soc.*, 1923, xlv, 1934. Ley and Arends, *Ber. deutsch. chem. Ges.*, 1928, 618, 212.

[2] Ling and Nanji, *Biochem. J.*, 1922, xvi, 707.

Group I. Each sample was photographed without any preliminary treatment except drying and grinding. The spectra of samples A, B, and C were exactly the same. The spectrum of sample D was different.

Group II. A part of each sample was dissolved in water and the crystals separated by slow evaporation to dryness. This produced the plate form of the crystals. The diffraction patterns were identical, and the same as the spectra of samples A, B, and C of group I. The difference observed in sample D of group I had disappeared.

Group III. A part of each sample of group I was dissolved in water and the needle shaped crystals formed by adding absolute alcohol. All 4 spectra of this group were alike but slightly different from the spectra of Group I. With more complete drying, however, a change took place in the crystals with the result that the spectra became identical with those of Group II. The 3 types of spectrum are shown in Figure I.

We may conclude from these data that there are at least 2 crystalline forms of glycine, the crystals of Group III being different

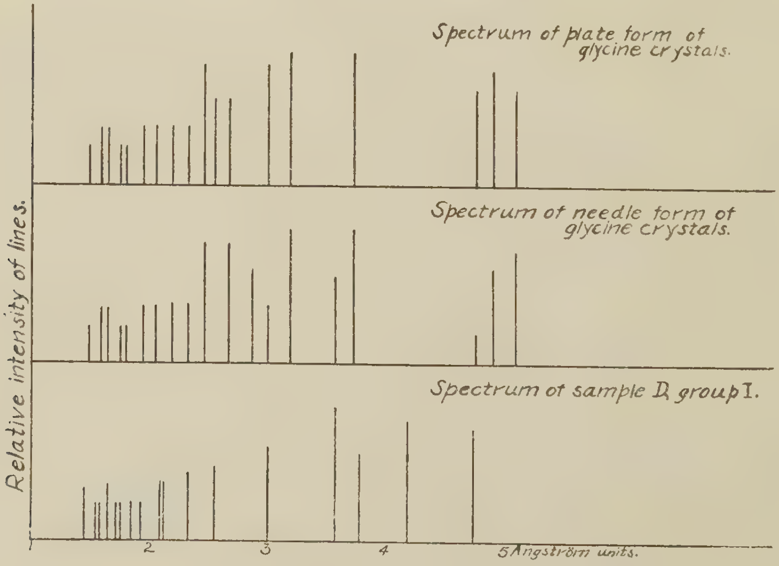


FIG. 1.

Three diffraction spectra of glycine. Calculated spacings between atomic planes are plotted on the abscissae and relative intensities of the lines are indicated by the length of the corresponding ordinates.

from the others, possibly because of containing alcohol of crystallization. These crystals readily change, as a result of prolonged drying, so that their crystalline structure is the same as that of the plate form. These results do not indicate, however, that there is any difference in the structure of the glycine molecule in the plate and needle forms of the crystals. At present the spectrum of sample D, Group I, cannot be explained.

Work is being done in an attempt to elucidate the complete crystal structure of glycine.

4382

Changes in the Size of the Spleen Following Exercise.

S. F. COOK AND M. I. ROSE.

From the Department of Physiology, University of California, Berkeley.

Barcroft and Stephens[1] in a recent investigation have shown that gross changes in the size of the mammalian spleen may be studied in so called exteriorized preparations by means of outline tracings on a plate of celluloid. They find that during muscular activity the spleen shrinks to approximately two-thirds of its resting area.

It seemed worth while to us to investigate the course of recovery in the size of spleens which had contracted as a result of periods of exercise of widely varying intensity and duration. The technique of our experiments differed from that of the Cambridge workers in that we were making recovery observations over short periods of time on decerebrate cats, exercised by means of electrical stimulation. Hence it was sufficient to make a temporary exteriorization which consisted essentially of bringing the spleen through a 2 inch incision which had been carried from the skin through the peritoneum. During active exercise and when no actual measurements were being made, the spleen was returned to its place in the abdomen, and the incision was temporarily closed. Control experiments showed that this repeated exteriorization and replacement *per se* had no apparent effect on size. The area of the spleen was computed from the tracings and used as a basis of comparison.

The results of our experiments show that in cats which were decerebrated so that no rigidity developed, the spleen immediately after exercise had contracted to about 70% of its original area; the latter had been determined before exercise. Our experiments on this point thus check with those of Barcroft and Stephens within reasonable limits.

---

[1] Barcroft, J., and Stephens, J., *J. Physiol.*, 1927, lxiv, 1.

Having measured the extreme contraction immediately after exercise, we then traced the course of recovery by continuing measurements until the spleen had attained all or most of its resting size. We found that recovery begins as soon as the exercise ceases because our measurements (made within 1 minute of the cessation of the electrical stimulation) invariably show a spleen which is increasing in size. This increase appears to consist of 2 phases, of which the first is very rapid, particularly within the first 5 minutes. The second is slower, continuing until the spleen has returned to its resting size. This usually takes from 15 to 30 minutes.

The speed of the first, or rapid, phase of recovery appears to be nearly constant under all conditions, and is independent of the intensity or duration of the preceding exercise. When the data are plotted as per cent of normal size, against time, the slope of the resulting curves for the first 3 to 5 minutes is constant within the error of the method. The speed of the second, slower, phase of recovery is variable and our data have not as yet enabled us to relate it definitely to any of the experimental conditions.

A few cats were not decerebrated but were anesthetized with amytal. The same 2 phase recovery was here likewise observed.

4383

Ultrafiltration Studies on the Bacteriophage.

A. P. KRUEGER AND H. T. TAMADA. (Introduced by E. W. Schultz.)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Studies on the magnitude of the bacteriophage corpuscle have been carried out by a number of investigators with widely different result. Thus Bechhold and Villa,[1] Prausnitz,[2] and Stassano and de Beaufort[3] assigned a diameter of about 20$\mu\mu$ to the lytic particle; Eliava and Suarez[4] conclude that the diameter is considerably less than $20\mu\mu$, while Zinsser and Tang[5] place it within the range from $20\mu\mu$ to $100\mu\mu$.

---

[1] Bechhold, H., and Villa, L., *Z. Hyg. u. Infektionskrankh*, 1925-26, cv, 601.

[2] Prausnitz, C., *Klin. Woch.*, 1922, i, 1639.

[3] Stassano, H., and de Beaufort, A. C., *Compt. rend Soc. de Biol.*, 1925, xciii, 1378.

[4] Eliava, G., and Suarez, E., *Compt. rend. de Soc. de Biol.*, 1927, xcvi, 462.

The mechanism of ultrafiltration, a procedure usually employed in making such determinations, has recently been critically studied by Hitchcock,[6, 7, 8] Grollman[9] and others, who emphasize the necessity of conducting experiments of this kind under as simple and well controlled conditions as possible.

In ultrafiltration studies on the bacteriophage more ideal experimental conditions are undoubtedly realized by employing protein-free suspensions, a procedure made possible by the work of Arnold.[10] Arnold devised a method based upon the diffusion of the lytic principle into agar whereby one may prepare highly active bacteriophage suspensions which neither give any chemical reaction for proteins, nor, when injected into animals, produce antibodies directed against the proteins normally associated with the bacteriophage.

The question of the magnitude of protein-free bacteriophage is of particular interest because of Bronfenbrenner's[11] conclusion that the substance responsible for the lytic action of bacteriophage is adsorbed on colloidal particles of various sizes. We decided, therefore, to undertake ultrafiltration studies for the purpose of eliciting any difference in particle size between purified (protein-free) and non-purified bacteriophage.

A highly virulent race of anti-coli bacteriophage (Phage B) was used in our experiments and was propagated exclusively on one strain of *B. coli* (k-13). The non-purified lysate in Martin's broth was adjusted to the same lytic titre as that of the purified phage extracted from agar according to the method of Arnold. These latter extracts were consistently protein-free when tested with the ordinary chemical tests for proteins. The pH of purified and non-purified suspensions varied between pH 6.0-7.6 within which range we have satisfied ourselves, by cataphoresis experiments, that both types of preparation contained lytic particles bearing a negative charge.

The membranes employed were made by impregnating Whatman No. 1 filter paper with glacial acetic acid containing various percentages of dried Anthony's negative cotton and were standardized with colloidal suspensions of predetermined particle size as well as by their permeability to water (Poisseuille's Law). In the following work "percentage" of a membrane refers to the weight of dried negative cotton per 100 gm. of glacial acetic acid used in its preparation. Filtration pressures varied between 10 and 20 mm. of mercury.

---

[5] Zinsser, H., and Tang, F., *J. Exp. Med.*, 1927, xlvi, 357.

[6] Hitchcock, D. I., *J. Gen. Physiol.*, 1925-27, viii, 61.

[7] Hitchcock, D. I., *J. Gen. Physiol.*, 1926-27, x, 179.

[8] Hitchcock, D. I., *J. Gen. Physiol.*, 1925-26, ix, 755.

[9] Grollman, A., *J. Gen. Physiol.*, 1925-26, ix, 813.

[10] Arnold, L., *J. Lab. and Clin. Med.*, 1923, viii, 720.

[11] Bronfenbrenner, J., *J. Exp. Med.*, 1927, xlv, 873.

The following experimental results were obtained consistently in repeated tests to date: 1. Purified phage B. passes a 4.5% or 5% membrane without diminution in titre. The identical membrane, washed free of the lytic principle with 10-20 cc. portions of sterile distilled water, completely retains non-purified Phage B. 2. The sequence may be reversed. A 4.5% membrane completely retains non-purified Phage B. The identical membrane, without intermediate washing, passes purified Phage B quantitatively. This same result is obtained with 5% membranes. 3. Purified Phage B. added to water containing very small amounts of Martin's broth or bacterial autolysates, is completely retained by a 4.5% and 5% membranes. The identical membranes permit purified Phage B. not so treated to pass quantitatively. 4. Increased disperson up to one volume of non-purified Phage B. with 10 volumes of distilled water or Martin's broth does not render the non-purified phage more readily filterable. Thus, non-purified Phage B. is retained as usual by a 4% or 5% membrane. The same membrane retains Phage B. diluted with 10 volumes of distilled water or Martin's broth, but is permeable to purified Phage B.

Our data thus far indicate that protein-free bacteriophage is definitely smaller than ordinary, non-purified bacteriophage. The non-purified bacteriophage aggregate does not appear to be strongly adsorbed by the membranes, for merely washing with water removes it readily. Neither does non-purified bacteriophage alter detectably the effective pore size of retaining membranes, for the identical membranes which retain non-purified bacteriophage, pass purified bacteriophage without reduction of lytic titre, and further, give no evidence of reduced pore size when subjected to the usual tests (retention of colloidal particles of known magnitude, permeability to water, pressure required to force air through the moist membrane). Apparently the purified bacteriophage readily forms aggregates with protein or protein derivative contained in broth or bacterial autolysates, these aggregates possessing larger magnitudes than the purified protein-free bacteriophage itself. Increased dispersion in water or in Martin's broth does not appear to render the non-purified bacteriophage more filterable.

Preliminary experiments with denser membranes tend to place the diameter of the purified bacteriophage in the neighborhood of 5.0 milli-microns. The detailed results of this work will be reported in another paper upon completion.

4384

Phosphocreatine in Insulinized Frogs.

J. M. D. OLMSTED AND H. MACKLER.

From the Department of Physiology, University of California.

Fiske and Subbarow[1] have isolated phosphocreatine from protein-free muscle filtrates and find "that the compound is destroyed during contraction at a rate which rivals that of glycogenolysis and lactic acid production." Modern theories of muscle contraction have as their foundation the change of glycogen into lactic acid. But it has been shown that the muscles of insulinized frogs can contract in a normal manner even if no glycogen can be found in them.[2]

To determine whether there is a correlation between glycogen and phosphocreatine we have analyzed individual gastrocnemii of very large frogs for initial phosphorus, phosphocreatine, total phosphorus, lactacidogen, glycogen, and lactic acid.

One gastrocnemius was removed with as little stimulation as possible, frozen in liquid air, and weighed portions (0.5 gm. or more) were taken for each of the different estimations. The other gastrocnemius was removed at the same time and stimulated at second intervals nearly to exhaustion, then frozen, portions weighed, etc.

In normal frogs we found 6-20 mgm. of phosphocreatine per gm. of tissue in resting muscles, and very little or none in exercised muscles. Changes in lactacidogen (*i. e.*, total phosphorus minus initial phosphorus and phosphocreatine) were too irregular for any definite conclusions to be drawn from the data. The glycogen content of resting muscle was 6-8 mgm. per gm. of tissue, and the loss of glycogen in the exercised muscles, 1-2 mgm., was approximately balanced by the gain in lactic acid. Normal resting muscles are, therefor, high both in phosphocreatine and glycogen, and worked muscles lose all their phosphocreatine and much of their glycogen

In insulinized frogs which had had violent convulsions for several days we found the following conditions: The phosphocreatine content of resting muscles was always low, many times being entirely absent. Glycogen was also low in these muscles, in many cases being undetectable. Yet the muscles were able to do approximately their normal amount of work in lifting weights. In the cases where the resting muscle was glycogen free, its worked fellow showed an increase in lactic acid comparable to that in normal worked muscles. In spite of the general statement that in the resting insulinized muscles there was little phosphocreatine and little glycogen, there is really no direct relation between them, since in some cases there was no phosphocreatine but a considerable amount of glycogen, and vice versa. This irregularity is illustrated by 2 cases: in one 1.7 mgm. of glycogen was found and there was no phosphocreatine; in the other 1.7 mgm. of glycogen was again found, but this time there was 12.8 mgm. of phosphocreatine present.

---

[1] Fiske, C. H., and Subbarow, Y., *Science*, 1928, lvii, 169.

[2] Olmsted, J. M. D., and Harvey, J. M., *Am. J. Physiol.*, 1927, lxxx, 643.

Phosphocreatine and glycogen, although low or absent in insulinized muscle, vary independently of each other and furthermore, contraction can take place when either one or the other, or both are lacking.

4385

Occurrence of Coccidioides Immitis in Lesions of Slaughtered Animals.

M. DORTHY BECK.* (Introduced by W. H. Kellogg.)

From the California State Department of Public Health.

During the course of an epidemiological investigation of *coccidioidal granuloma*, examinations were made of lesions from slaughtered animals. Specimens were obtained through the cooperation of the Bakersfield Packing Company, Kern County, California. A total of 38 such specimens were examined over a period of 3 months. The following table gives the details.

Six of the positives were from cattle and one was from a sheep. The lesions seem to be localized in the upper respiratory tract and apparently no other lesions are to be found in the animals. Whether the disease would become generalized in time cannot be stated as there is no evidence available at present. A study of the histories

---

* Acknowledgement is made of the courtesies extended by Doctor Joe Smith, Health Officer of Kern County, California, in providing laboratory facilities for carrying on this investigation in the field.

TABLE I.

Showing Types of Lesions, Number Examined, with Number and Percentage Found Positive.

Type of Lesion in Slaughtered Animal.	Number Examined	Number Positive	% Positive
Skin abscess	1	0	0
Sub-maxillary gland	5	0	0
Mediastinal lymph gland	7	5	71.4
Bronchial lymph gland	5	2	40.0
Retropharyngeal lymph gland	3	0	0
Lung	7	0	0
Liver	8	0	0
Mesenteric glands	1	0	0
Tongue abscess	1	0	0
Totals	38	7	18.4

of the infected animals reveals no common factor. The geographical distribution of these animals is as wide as that of the human cases recorded.

The method of examination was as follows: each specimen was given a serial number when brought to the laboratory. A direct smear of the pus was made using 10% KOH to demonstrate the presence of double contoured capsules. Another smear was made and stained by the acid-fast method for the presence of tubercle bacilli. This was done to eliminate tuberculosis. Some of the pus was stained with 1% hematoxylin. Cultures were made on Sabouraud's agar slants and in plain broth. These were incubated at 37.5° C. for 7 days. The average incubation for these strains was 4 days and the maximum was 6 days. The cultures were inoculated into guinea pigs as a final test in order to determine that the fungus growth was *Coccidioides immitis*. The autopsy specimens from these animals were examined by the same methods as above.

TABLE II.

Summary of Laboratory Examinations With Results.

Laboratory Number	Direct Smear	Acid-fast Stain	Hematoxylin Stain	Cultures	Sections of Original Lesions	Guinea Pigs (3)
9	Negative	Negative	D. C. C. (1)	Fungus in 5 days	Positive	Positive
14	"	"	(2)	" " 4 "	(2)	"
18	"	"	D. C. C. (1)	" " 3 "	(2)	"
19	"	"	" (1)	" " 3 "	Positive	"
22	"	"	" (1)	" " 6 "	"	"
26	D. C. C. (1)	"	" (1)	" " 4 "	"	"
29	"	"	" (1)	" " 3 "	(2)	"

(1) Double contoured capsules.
(2) Insufficient material, *i. e.*, pus on swab.
(3) Cultures and smears from lesions positive.

Through the help and cooperation of Doctor E. C. Dickson, Stanford Medical School, San Francisco, sections were made of the original animal lesions and also of the infected guinea pigs. The results confirmed the cultural findings. Table II is a summary of the laboratory examinations made, with the results.

It may be concluded from the above table that the lesions from 7 slaughtered animals, *i. e.,* 6 cattle and 1 sheep were proven to contain *Coccidioides immitis.* Giltner[1] encountered the infection in bovine bronchial and mediastinal lymph glands from an animal slaughtered in San Diego, California. Although very little is known of the mode of infection of *coccidioidal granuloma* it does not seem likely that humans are infected from animals; but, rather that both man and animals are infected from the same source.

4386

Experiments with Reference to the More Heat-stable Factor of the Vitamin B Group (Factor P-P, Vitamin B_2 or G).*

H. C. SHERMAN AND M. R. SANDELS.

From the Department of Chemistry, Columbia University, New York.

Sparing solubility of this substance in alcohol. Extraction experiments with 80% alcohol were deemed of special interest because of the use of such alcohol extracts by Goldberger and associates[1] and because alcohol of almost the same concentration (79%) had been used by Osborne and Wakeman[2] for the precipitation of their "fraction II" which contained a relatively high concentration of both of the recently differentiated water-soluble factors required in the growth of rats.

In the present experiments, 400 gm. of air-dry baker's yeast were treated with 1500 cc. of alcohol (80% by weight), thoroughly stirred, and allowed to stand at room temperature (20°-25° C.) for 24 hours; then filtered with suction, and the yeast washed on a Büchner filter with 750 cc. of alcohol of the same strength; then again stirred with 1500 cc. of the alcohol, allowed to stand 24 hours,

---

[1] Giltner, L. T., *J. Agr. Research,* 1928, xiv, 533.

* Published as contribution No. 598, Department of Chemistry, Columbia University.

[1] Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *United States Public Health Reports,* 1926, xli, 297.

[2] Osborne, T. B., and Wakeman, A. J., *J. Biol. Chem.,* 1919, xl, 383.

filtered and washed as before. The residue was dried in the air at room temperature.

The extract obtained by combining the 2 filtrates and the washings was a clear yellow solution of pH 6.1. It was concentrated on the steam bath and evaporated at room temperature on cornstarch. Feeding experiments indicated that this extract contained the greater part of the antineuritic vitamin but scarcely measurable amounts of the heat-stable factor. The extracted residue, however, was somewhat less potent than the original yeast as a source of this latter factor.

Extraction as above with alcohol 60% (by weight) gave a greenish yellow-brown solution of pH 5.9, which frothed easily. Feeding experiment with the extracted residue indicated that it retained about one-half of the heat-stable factor of the dry yeast. The evaporated extract, while showing decided activity, seemed less potent than the extracted residue.

Similar extraction with alcohol of 95% (by weight) yielded a very pale solution of pH 6.4 which contained about 7% of the solids of the yeast but not enough of this heat-stable factor (vitamin B_2 or G) for detection by the method here used. The extracted residue seemed as potent as the original yeast.

The experiments here reported suggested the possibility of some destruction of the heat-stable factor, perhaps through oxidation, during the preparation and drying of the alcohol extracts.

More detailed studies of alcohol extraction and of the quantitative determination of vitamins B_1 and B_2 (the anti-neuritic and the more heat-stable factors) are in progress in this laboratory.

General plan of feeding experiments. Healthy young albino rats were placed when 28-30 days old upon a basal diet adequate in other respects but freed as completely as was here practicable from the heat-stable factor to be studied; that is, upon the Sherman and Spohn[3] "vitamin B-free" basal diet (using this expression in the older sense) which had been modified by the addition of an alcoholic extract of ground whole wheat previously proven to furnish an adequate supply of the antineuritic vitamin. In this way the heat-stable substance (although possibly supplied in small amounts) became the sole growth-limiting factor, so far as present knowledge indicates.

The rats were kept upon the basal diet alone until growth had ceased and a slight loss in weight had occurred. Following this depletion period, they received the basal diet with and without quantitatively graded additions of the original dry yeast and of the various extracts and residues prepared from it as indicated above. In order to free these extracts completely from alcohol and bring them into suitable form for quantitative feeding experiments, they were first concentrated on the steam bath and then evaporated upon cornstarch at room temperature. Quantitatively graded allowances of these preparations were fed three times weekly, separately from the basal diet.

---

[3] Sherman, H. C., and Spohn, A. A., *J. Am. Chem. Soc.*, 1923, xlv, 2719.

Effects of limitation of intake of this vitamin upon the weight curve and condition of the experimental animal. As in the case of the vitamin B complex and of the antineuritic factor so also of the more heat-stable factor, the feeding of graded amounts, as supplements to the basal diet, resulted in a series of weight curves graded from those of the "negative controls" to those approaching a normal rate of growth. In other words, the limitation of growth or the loss of body weight was somewhat proportional, though not in simple arithmetical proportion) to the severity of the restriction of intake to this factor (vitamin B_2 or G).

In respect to this observation and those which follow, our findings confirm and extend the work of previous investigators and, we believe, add somewhat to the definiteness and precision of our knowledge of the nutritional significance of this factor.

When this factor (P-P, vitamin B_2 or G) is excluded almost (though possibly not quite) completely from a food intake adequate in other respects, in experiments with rats of an initial age of 4 weeks, growth is quickly retarded and a loss of body weight may begin within 2 weeks; but this time interval is subject to considerable variation depending upon the completeness with which the diet has been freed from the factor in question, and, we believe, also upon the previous diet of the animal. Several weeks later, soreness of the eyes appears with abnormal secretion and a tendency to sticky lids, and to frequent rubbing and occasional scratching of the lids by the animal; in many cases the fur then falls out around the eyes, leaving bald inflamed rims; usually the mouth becomes sore, first with indications of excessive salivation, then with inflammation at the corners, sometimes progressing to an inflamed or ulcerated condition of the entire lower lip; in many cases, though less regularly, soreness of the nose develops with occasional bleeding; the animal becomes weak and tends to remain in a humped posture; in the advanced stages of this deficiency disease, diarrhea is common and there may be bloody discharges, both in feces and urine. When these cases of almost complete deprivation of vitamin B_2 or G were allowed to run a fatal course, death sometimes occurred without the development of conspicuous skin symptoms, though the fact that an abnormal condition of the skin had actually developed was indicated by extensive desquamation occurring in those instances in which life was prolonged or health restored by curative feedings.

In general our experiments indicated that in the condition resulting from almost complete deprivation of this vitamin the eyes, nose, mouth, alimentary tract, and perhaps kidneys are apt to show more pronounced abnormalities than the skin; whereas with a less complete deprivation and a somewhat longer course of the deficiency disease the abnormalities of the skin are apt to become much more prominent.

In these latter less rapidly fatal cases, the fur becomes dry and pulls out readily. Often a definite saddle-like pattern appears on the fur of the back. An examination of the area affected may reveal small dry cream-colored scales on the skin, which later may be replaced by larger yellowish crusts, appearing on the sides of the back, the shoulders, and the chest. The skin lesions usually show a tendency to symmetry; there are occasional cases of rough, red areas upon the paws and the inside of the fore legs; there may be more or less pronounced development of other symptoms, such as have just been mentioned as characteristic of the more acute cases.

On the whole, the symptoms observed by us are sufficiently consistent with those reported by Goldberger and his coworkers and by Chick and Roscoe[4] to justify the statement that the experimental reproducibility in rats of the conditions called pellagra-like by those investigators has been abundantly confirmed. Whether or not future usage will apply the term antipellagric to it, we would emphasize the fact that this heat-stable vitamin (B_2 or G) is evidently a substance of coördinate importance with the longer-known vitamins as an essential factor in normal nutrition and that deprivation or serious shortage of this substance results in widespread injury to the body tissues. Conversely, the liberal feeding of this substance may be expected to play a significant part in inducing a better-than-average nutritive condition—a problem which is being approached from various angles in the experimental work of this laboratory.

Curative experiments. Some of the animals showing marked symptoms of the dietary deficiency here described have been cured by the feeding of materials selected as rich in vitamin B_2 or G, without other change in their diet and without any change in sanitary conditions. These curative experiments yielded such uniformly clear-cut results as to furnish strong confirmation, if such were needed, of the view that the condition in question is essentially a dietary deficiency disease in the sense in which this term is ordinarily used by students of nutrition, although, as in the case of vitamin A, the typical clinical picture may include the results of infection or infections, the incidence and development of which become prominent under the conditions of the nutritional deficiency.

---

[4] Chick, H., and Roscoe, M. H., *Biochem. J.*, 1927, xxi, 698.